NTP
National Toxicology Program
U.S. Department of Health and Human Services

NTP Technical Report on the Toxicity Studies of Sodium Metavanadate (CASRN 13718-26-8) and Vanadyl Sulfate (CASRN 27774-13-6) Administered in Drinking Water to Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats and B6C3F1/N Mice

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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 Toxicity Report series began in 1991. The studies described in the NTP Toxicity Report series are designed and conducted to characterize and evaluate the toxicological potential of selected substances in laboratory animals (usually two species, rats and mice). Substances (e.g., chemicals, physical agents, and mixtures) selected for NTP toxicity studies are chosen primarily on the basis of human exposure, level of commercial production, and chemical structure. The interpretive conclusions presented in the toxicity reports are derived solely from the results of these NTP studies, and extrapolation of these 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 toxic potential.

NTP conducts its studies in compliance with its laboratory health and safety guidelines and 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.

NTP Toxicity 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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(1) Peer review the draft *NTP Technical Report on the Toxicity Studies of Sodium Metavanadate and Vanadyl Sulfate Administered in Drinking Water to Sprague Dawley (Hsd:Sprague Dawley® SD®) Rats and B6C3F1/N Mice.*

(2) Comment on NTP's interpretations of the data.

NTP carefully considered reviewer comments in finalizing this report.

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Abstract

Oral human exposure to vanadium may occur due to its presence in food and drinking water and its use in dietary supplements. The most prevalent oxidation states of vanadium in food and drinking water have been characterized as tetravalent and pentavalent. Vanadyl sulfate and sodium metavanadate were selected as representative tetravalent (V^{4+}) and pentavalent (V^{5+}) test articles for these studies, respectively. To assess the potential for oral toxicity of vanadium compounds with differing oxidation states under similar test conditions, the 3-month National Toxicology Program (NTP) toxicity studies of sodium metavanadate and vanadyl sulfate were conducted in male and female Sprague Dawley (Hsd:Sprague Dawley® SD®) rats (including perinatal exposure) and in B6C3F1/N mice. Drinking water concentrations for sodium metavanadate (0, 31.3, 62.5, 125, 250, and 500 mg/L) and vanadyl sulfate (0, 21.0, 41.9, 83.8, 168, and 335 mg/L) were selected on the basis of previously published 14-day drinking water studies conducted as part of the NTP vanadium research program.

During the perinatal phase of the rat sodium metavanadate study, dams and pups exposed to the highest concentrations had lower survival. Moribund dams were removed beginning on gestation day 22, and select dams and pups continued to be removed due to moribundity or mortality throughout the lactation period. These removals collectively resulted in four litters in the 500 mg/L sodium metavanadate group available to populate the postweaning study. There were no effects on survival of vanadyl sulfate-exposed dams or pups.

In the perinatal and 3-month rat studies, 10 F_1 pups/sex/group were selected for continuation during the postweaning phase; all available pups (n = 12 or 13) were retained in the 500 mg/L group for the sodium metavanadate study. In the 3-month mouse studies, 10 animals per sex were assigned to each exposure group. Lower water consumption was observed in both rats and mice at the highest exposure concentrations for both test articles. Lower body weights were observed at the end of the 3-month studies in rats and mice exposed to sodium metavanadate and in mice exposed to vanadyl sulfate. Absolute thymus weights were decreased in male and female mice exposed to sodium metavanadate. Several other organ weight changes occurred in these studies and were considered secondary to body weight changes.

For rats and mice exposed to sodium metavanadate and mice exposed to vanadyl sulfate, hematological effects related to erythrocyte microcytosis, including decreased mean cell volume, were consistently observed. This response was more severe in mice, with corresponding reductions in hematocrit and hemoglobin as well as an erythrocytosis response, including increased erythrocytes and reticulocytes. In sodium metavanadate-exposed rats, decreased globulin and cholesterol were observed. Epithelial hyperplasia of the ileum was observed in rats and mice exposed to sodium metavanadate and vanadyl sulfate. This lesion was not observed in any control animals. Other sites of the small or large intestine, including the jejunum, were observed to have epithelial hyperplasia, but these observations were not consistent across test articles or sex/species.

Vanadium intake was calculated using the amount of vanadium in each test article (41.7% in sodium metavanadate and 31% in vanadyl sulfate), the exposure concentration, and the measured water consumption. In rats, the overall lowest-observed-effect levels (LOELs) for both sodium metavanadate and vanadyl sulfate align well with vanadium intake, indicating that total vanadium, rather than vanadium oxidation state, may be a driver for toxicity. In mice, however,

the overall LOELs for both compounds and the LOELs for specific endpoints do not occur at the same calculated vanadium doses between test articles.

Sodium metavanadate and vanadyl sulfate were not mutagenic in several bacterial tester strains, with or without exogenous metabolic activation (S9 mix), and no biologically significant increases in micronucleated reticulocytes were seen in male and female rats and mice. An increase in the percentage of circulating immature erythrocytes (reticulocytes) was seen in male rats and in male and female mice when exposed to sodium metavanadate, whereas there were no changes in this population of cells in female rats. When exposed to vanadyl sulfate, increases in the percentage of reticulocytes were seen in male and female mice; however, no notable changes in this population of cells were observed in male and female rats.

Under the conditions of these studies, oral exposure to sodium metavanadate or vanadyl sulfate in drinking water resulted in hematological effects associated with erythrocyte microcytosis in rats (sodium metavanadate) and mice (sodium metavanadate and vanadyl sulfate), including erythrocytosis in male rats and in male and female mice. Epithelial hyperplasia of the ileum and other gastrointestinal sites was observed histologically, which was consistent for both test articles and across both sexes and species evaluated. In the sodium metavanadate studies, the LOELs were 125 mg/L in male and female rats, 31.3 mg/L in male mice, and 62.5 mg/L in female mice, based on changes in hematology (male rats and male and female mice) and epithelium hyperplasia in the ileum and jejunum (male and female rats). In the vanadyl sulfate studies, the LOELs were 168 mg/L in male and female rats and 83.8 mg/L in male and female mice, as indicated by epithelium hyperplasia in the ileum (male and female rats and mice) and hematology (female mice).

Synonyms for sodium metavanadate: sodium (meta)vanadate; sodium vanadate(V); vanadic acid, monosodium salt; sodium trioxovanadate; sodium vanadium oxide; monosodium trioxovanadate(1-); sodium vanadate; sodium trioxidovanadate(1-); sodium vanadium trioxide; vanadic acid (HVO_3), sodium salt (8Cl)

Synonyms for vanadyl sulfate: vanadium, oxo(sulfato(2-)-O)-; vanadium oxide sulphate; vanadic sulfate; oxovanadium(2+) sulfate; oxovanadium(IV) sulfate; vanadium(IV) oxysulfate; (oxido)vanadium(2+) sulfate; oxosulfatovanadium(IV); oxovanadium(IV) sulfate; vanadium oxide sulfate; vanadium oxosulfate; vanadium oxysulfate; vanadium sulfate; vanadyl monosulfate; vanadin(IV) oxide sulfate

Summary of Findings Considered Toxicologically Relevant in Male and Female Rats and Mice Exposed to Sodium Metavanadate or Vanadyl Sulfate in Drinking Water for Three Months

	Male Sprague Dawley Rats	Female Sprague Dawley Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Concentrations in Drinking Water				
Sodium Metavanadate	0, 31.3, 62.5, 125, 250, or 500 mg/L	0, 31.3, 62.5, 125, 250, or 500 mg/L	0, 31.3, 62.5, 125, 250, or 500 mg/L	0, 31.3, 62.5, 125, 250, or 500 mg/L
Vanadyl Sulfate	0, 21.0, 41.9, 83.8, 168, or 335 mg/L	0, 21.0, 41.9, 83.8, 168, or 335 mg/L	0, 21.0, 41.9, 83.8, 168, or 335 mg/L	0, 21.0, 41.9, 83.8, 168, or 335 mg/L
Survival Rates				
Sodium Metavanadate	15/15, 15/15, 15/15, 15/15, 15/15, 12/13	15/15, 15/15, 15/15, 15/15, 15/15, 10/12	No effect[a]	No effect[a]
Vanadyl Sulfate	No effect	No effect	No effect	No effect
Body Weights				
Sodium Metavanadate	F_1 generation: *Lactation:* ↓ (125, 250, 500 mg/L groups: up to 21% lower than the control group) *Study termination:* ↓ (125, 250, and 500 mg/L groups: up to 27% lower than the control group)	F_0 generation: *Gestation:* ↓ (125, 250, and 500 mg/L groups: up to 14% lower than the control group) *Lactation:* No effect F_1 generation: *Lactation:* ↓ (500 mg/L group: 22% lower than the control group) *Study termination:* ↓ (500 mg/L group 11% lower than the control group)	↓ (250 and 500 mg/L groups: 11% and 26% lower than the control group, respectively)	↓ (250 and 500 mg/L groups: 14% and 23% lower than the control group, respectively)
Vanadyl Sulfate	F_1 generation: *Lactation:* Exposed groups within 10% of the control group *Study termination:* Exposed groups within 10% of the control group	F_0 generation: *Gestation:* Exposed groups within 10% of the control group *Lactation:* No effect F_1 generation: *Lactation:* Exposed groups within 10% of the control group *Study termination:* No effect	↓ (168 and 335 mg/L groups: 10% and 13% lower than the control group, respectively)	No effect
Clinical Findings				
Sodium Metavanadate	Ruffled coat, hunched, lethargy	Ruffled coat, hunched, lethargy	None[b]	None
Vanadyl Sulfate	None	None	None	None

	Male Sprague Dawley Rats	Female Sprague Dawley Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Water Consumption				
Sodium Metavanadate	250 and 500 mg/L groups: 11% and 16% lower than the control group, respectively	250 and 500 mg/L groups: 19% and 28% lower than the control group, respectively	500 mg/L group: 15% lower than the control group	500 mg/L group: 15% lower than the control group
Vanadyl Sulfate	83.8, 168, and 335 mg/L groups: up to 21% lower than the control group	335 mg/L group: 24% lower than the control group	335 mg/L group: 10% lower than the control group	21.0, 41.9, 83.8, 168, and 335 mg/L groups: up to 17% lower than the control group
Organ Weights				
Sodium Metavanadate	No effect	No effect	↓ Absolute thymus weight	↓ Absolute thymus weight
Vanadyl Sulfate	No effect	No effect	No effect	No effect
Nonneoplastic Effects				
Sodium Metavanadate	Small intestine: ileum, epithelium, hyperplasia (0/10, 0/10, 0/9, 5/9, 9/10, 12/13); jejunum, epithelium, hyperplasia (0/10, –[c], 0/9, 1/9, 6/10, 7/13)	Small intestine: ileum, epithelium, hyperplasia (0/9, –, 0/10, 4/10, 9/10, 12/12); jejunum, epithelium, hyperplasia (0/9, –, –, 1/10, 0/10, 6/12)	Small intestine: ileum, epithelium, hyperplasia (0/10, –, 0/10, 2/10, 5/10, 7/10)	Small intestine: ileum, epithelium, hyperplasia (0/10, –, 0/10, 2/10, 9/10, 8/10)
Vanadyl Sulfate	Small intestine: ileum, epithelium, hyperplasia (0/10, –, –, –, 3/10, 9/10)	Small intestine: ileum, epithelium, hyperplasia (0/10, –, 0/10, 0/10, 4/10, 9/10)	Small intestine: ileum, epithelium, hyperplasia (0/10, –, 0/9, 2/9, 3/10, 9/10)	Small intestine: ileum, epithelium, hyperplasia (0/10, –, 0/10, 1/9, 5/10, 10/10)
Hematology				
Sodium Metavanadate	↑ Erythrocytes ↓ Mean cell volume ↓ Mean cell hemoglobin concentration	↑ Erythrocytes ↓ Mean cell volume ↓ Mean cell hemoglobin concentration	↑ Erythrocytes ↑ Reticulocytes ↓ Mean cell volume ↓ Hematocrit ↓ Hemoglobin	↑ Erythrocytes ↑ Reticulocytes ↓ Mean cell volume ↓ Hemoglobin
Vanadyl Sulfate	None	None	↑ Reticulocytes ↓ Mean cell volume ↓ Mean cell hemoglobin concentration ↓ Hemoglobin	↑ Erythrocytes ↓ Mean cell volume ↓ Mean cell hemoglobin concentration
Clinical Chemistry				
Sodium Metavanadate	↓ Globulin ↓ Cholesterol	↓ Globulin ↓ Cholesterol	–	–

	Male Sprague Dawley Rats	Female Sprague Dawley Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Vanadyl Sulfate	No effect	No effect	–	–
Reproductive Findings				
Sodium Metavanadate	No effect	No effect	No effect	No effect
Vanadyl Sulfate	No effect	No effect	No effect	No effect
Pubertal Endpoints				
Vaginal Opening				
Sodium metavanadate	–	Delayed (250 mg/L)	–	–
Vanadyl sulfate	–	Delayed (335 mg/L)	–	–
Genetic Toxicology				
Bacterial Mutagenicity		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		Sodium metavanadate: negative in males and females Vanadyl sulfate: negative in males and females		
Mouse peripheral blood		Sodium metavanadate: negative in males and females Vanadyl sulfate: negative in males and females		

[a]One male and one female mouse exposed to 500 mg/L and 250 mg/L sodium metavanadate, respectively, died during the study; however, these deaths were not considered exposure related.
[b]None = no toxicologically relevant effects for this endpoint.
[c]Data were not collected for an exposed group when animals exposed to higher concentrations exhibited no incidences of nonneoplastic lesions in that tissue.

Overview

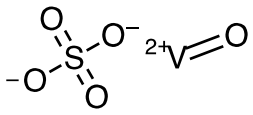
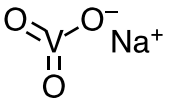
The potential adverse effects of various vanadium compounds were previously assessed in National Toxicology Program (NTP) studies. Vanadium pentoxide is commercially produced and imported for use as an industrial intermediate in the production of vanadium and steel alloys as well as for other industrial purposes. Concern about human exposure to vanadium pentoxide is primarily in the occupational setting via inhalation. The results of NTP toxicity and carcinogenicity studies in F344/N rats and B6C3F1 mice following whole-body inhalation exposure to vanadium pentoxide for 16 days, 3 months, or 2 years were previously published.[1] A 28-day immunotoxicity study of sodium metavanadate in B6C3F1/N mice exposed via drinking water was also conducted as part of the NTP vanadium research program. Potential human oral exposure to vanadium compounds is likely due to its presence in food and drinking water from natural and anthropogenic sources and the use of vanadium-containing dietary supplements. Vanadium in food and drinking water can be found in various oxidation states with the most prevalent being tetravalent (V^{4+}) and pentavalent (V^{5+}). Vanadyl sulfate and sodium metavanadate were selected as representative tetravalent and pentavalent vanadium compounds, respectively, to assess the potential toxicity of vanadium oral exposure. To select exposure concentrations for the current studies, 14-day drinking water studies of vanadyl sulfate and sodium metavanadate were performed and are published.[2; 3]

Introduction

Chemical and Physical Properties

Vanadyl sulfate and sodium metavanadate are inorganic vanadium compounds with +4 (tetravalent, V^{4+}) and +5 (pentavalent, V^{5+}) oxidation states, respectively (Table 1). Vanadyl sulfate is a blue crystalline solid with a melting point of 265°C and is highly soluble in water (467 g/L at 20°C).[4] Sodium metavanadate is colorless to yellow in crystalline form or a cream-colored solid with a melting point of 630°C and a water solubility of 211 g/L at 25°C.[5-7]

Table 1. Vanadium Compounds Evaluated

Chemical Name	Chemical Formula	Oxidation State	CASRN	Molecular Weight (g/mol)	Structure
Vanadyl Sulfate[a]	$VOSO_4$	V^{4+}	27774-13-6	163.01	
Sodium Metavanadate[b]	$NaVO_3$	V^{5+}	13718-26-8	121.93	

[a]Synonyms: vanadium, oxo(sulfato(2-)-O)-; vanadium oxide sulphate; vanadic sulfate; oxovanadium(2+) sulfate; oxovanadium(IV) sulfate; vanadium(IV) oxysulfate; (oxido)vanadium(2+) sulfate; oxosulfatovanadium(IV); oxovanadium(IV) sulfate; vanadium oxide sulfate; vanadium oxosulfate; vanadium oxysulfate; vanadium sulfate; vanadyl monosulfate; vanadin(IV) oxide sulfate.
[b]Synonyms: sodium (meta)vanadate; sodium vanadate(V); vanadic acid, monosodium salt; sodium trioxovanadate; sodium vanadium oxide; monosodium trioxovanadate(1-); sodium vanadate; sodium trioxidovanadate(1-); sodium vanadium trioxide; vanadic acid (HVO_3), sodium salt (8Cl).

Production, Use, and Human Exposure

Vanadium is a naturally occurring element found in the earth's crust (approximately 100 ppm) and in a variety of mineral substances.[8] According to a study conducted in the 1980s, human exposure from background levels of vanadium in dietary sources is estimated at 6–18 µg/day (based on age group and sex) in the United States; young male populations are estimated to have the highest daily intake of 18.3 µg/day.[9] The primary forms of vanadium identified in food have been characterized and reported as vanadyl (V^{4+}) and vanadate (V^{5+}), likely because they are the more stable forms.[10] The contribution to background levels of vanadium in food is largely attributed to natural occurrences of the element, including weathering rock, soil erosion, and continental dust.

Vanadium also occurs naturally in coal and petroleum crude oils, which can lead to anthropogenic contributions to oral human exposure. The average concentration of vanadium in coal in the United States is approximately 30 ppm, with a range (15–34 ppm) determined by geographic origin.[8] By comparison, concentrations of vanadium in residual oil have a much larger range (1–1,400 ppm).[8] Residual oil is primarily used in oil-fired power plants and industrial boilers. Fly ash, a combustion product of coal and residual oil, contains vanadium and is a potential source of anthropogenic contamination of drinking water sources.[8]

In addition to natural and anthropogenic sources of vanadium that may lead to oral exposure in humans, the V^{4+} (including vanadyl sulfate) and V^{5+} (including sodium metavanadate) forms of vanadium are also sold in dietary supplements.[11] These supplements have purported benefits of improving glucose metabolism in diabetic patients, reducing hypertension and hyperlipidemia, and treating osteoporosis. Some of these uses are supported by mechanistic information for V^{4+} that indicate it is a protein tyrosine phosphatase inhibitor.[12]

Toxicity Assessments and Regulatory Status

In 2012, the Agency for Toxic Substances and Disease Registry (ATSDR) published a thorough review of the existing literature for health effects resulting from oral vanadium exposure (and other routes) in humans and experimental animals.[8] In studies of rats and mice orally exposed to sodium metavanadate or vanadyl sulfate, some findings were consistent across multiple studies. Hematological effects—including reduced hemoglobin, hematocrit, or erythrocytes and increased reticulocytes—were observed in two studies in Wistar rats exposed to sodium metavanadate.[13; 14]

Several studies in Wistar rats, Sprague Dawley rats, or Swiss mice exposed orally to either sodium metavanadate or vanadyl sulfate reported developmental and reproductive effects. Developmental effects included reduced pup body weight and survival,[15-17] and reproductive effects included reduced fertility and sperm counts.[18-20]

Increased blood pressure was observed in three studies of oral sodium metavanadate exposure in male Sprague Dawley rats.[19] Other cardiac effects included increased heart rate in male Sprague Dawley rats orally exposed to sodium metavanadate and reduced aorta diameter in male Swiss albino rats dosed via intraperitoneal injection with vanadyl sulfate.[21] Impaired neurobehavioral performance was observed in two studies of male Sprague Dawley rats orally dosed with sodium metavanadate.[22; 23]

Subsequent to this review, the results of 14-day drinking water studies of sodium metavanadate and vanadyl sulfate in adult rats and mice were published.[2; 3] The studies described in Roberts et al.[2] were conducted as part of the NTP vanadium research program and were important for the study design and concentration selection for the studies described here.

Elemental vanadium is included in the Contaminant Candidate List (CCL) of substances that do not have national regulatory guidelines and are known or anticipated to occur in public water systems.[24] Vanadium has been included on the CCL since its inception in 1998 with CCL1.[25] In 2020, the Environmental Protection Agency Integrated Risk Information System program initiated an assessment of vanadium and vanadium compounds following oral exposure.[26]

Absorption, Distribution, Metabolism, and Excretion

Experimental Animals

Interconversion between oxidation states and complex speciation under the conditions in the gastrointestinal (GI) tract and during transport in the systemic circulation makes investigating the absorption, distribution, metabolism, and excretion (ADME) behavior of vanadyl (V^{4+}) and vanadate (V^{5+}) compounds difficult. Higher absorption of vanadate compared to vanadyl has been reported due to differential speciation in the GI tract. For example, intestinal absorption of

the oxo-anions (e.g., H_2VO^{4-}, HVO_4^{2-}) of vanadate is known to be higher than that of the oxo-cations (e.g.,VO^{2+}) of vanadyl compounds.[27]

Limited ADME data available on vanadyl and vanadate compounds point to low absorption of vanadium following oral exposure in rodents; the absorbed dose is distributed into tissues with low retention and excreted via urine.[8; 27-30] Because speciation of vanadium using available analytical techniques is challenging, especially in biological matrices, data available are based on measurement of total vanadium. Following exposure of female Wistar rats to 100 ppm of sodium metavanadate in feed for 7 days, the majority of the dose was excreted in feces (83.5%), with small amounts recovered in urine (0.86%).[28] Vanadium was distributed to tissues, with higher concentrations found in bone, spleen, kidney, and liver relative to other tissues. Bogden et al.[29] reported similar findings following exposure of up to 25 ppm vanadium as sodium metavanadate via feed in female Sprague Dawley rats for 2 weeks; significantly higher levels were measured in plasma compared to erythrocytes.[29] In male Wistar rats, following exposure of 5 or 50 ppm vanadyl sulfate or sodium orthovanadate in drinking water for up to 3 months, vanadium concentrations increased in the kidney, liver, bone, muscle tissue, digestive tract, and blood over time and with increasing exposure concentrations.[30] Higher concentrations of vanadium were observed in tissues following exposure to sodium orthovanadate compared to vanadyl sulfate. The concentration of vanadium in all tissues except the bone declined rapidly after cessation of exposure, although sodium orthovanadate-exposed animals exhibited relatively high concentrations of vanadium, especially in the bone and kidney, 6 weeks following cessation of exposure.

In Sprague Dawley rats, the elimination half-life of vanadium in tissues following exposure to 160 μmol vanadium/kg body weight/day as vanadyl sulfate or sodium orthovanadate via a liquid diet for 1 week ranged from 3.18 to 15.95 days, with the kidney and liver having the shortest half-life (<4 days) and the testes having the longest (13.50–15.95 days).[31] In general, half-lives were slightly longer following administration of sodium orthovanadate compared to vanadyl sulfate. Ramanadham et al.[32] reported an elimination half-life of 11.7 days in the kidney following exposure to 0.75 mg/mL vanadyl sulfate trihydrate via drinking water in Wistar rats for 3 weeks.[32] Following a single gavage dose of 37.5 or 75 mg/kg vanadyl sulfate pentahydrate (7.56 or 15.12 mg vanadium/kg, respectively) in male Wistar rats, the estimated blood elimination half-life was approximately 173 hours (7.2 days); absolute bioavailability was approximately 16%.[33] Fetal transfer of vanadium has been reported following exposure of pregnant mice to vanadyl sulfate pentahydrate at doses ≤150 mg/kg/day from gestation day (GD) 6 through GD 15.[34]

Humans

Limited ADME data in the literature for vanadyl compounds suggest poor absorption of vanadium following oral exposure.[8; 27; 35; 36] Following ingestion of 50–125 mg ammonium vanadyl tartrate per day, <1% of the dose was excreted in urine within 24 hours.[35] Following daily oral dosing of diabetic patients with 25, 50, or 100 mg vanadium as vanadyl sulfate for 6 weeks, serum and blood vanadium concentrations were dose-proportional, and blood and serum elimination half-lives were between 4.6 and 5.6 days.[36]

Study Rationale

Vanadium was selected for evaluation via drinking water due to potential widespread oral human exposure and identified data collection needs,[8] which include comprehensive toxicological characterization and evaluation of test articles with different oxidation states under similar experimental designs and test settings. To address these information gaps, vanadyl sulfate and sodium metavanadate were selected as representative test articles for the tetravalent (V^{4+}) and pentavalent (V^{5+}) forms, respectively. Three-month drinking water studies were conducted with both test articles in Sprague Dawley (Hsd:Sprague Dawley® SD®) rats (including a perinatal phase) and B6C3F1/N mice.

Materials and Methods

Procurement and Characterization of Sodium Metavanadate and Vanadyl Sulfate

Sodium metavanadate was obtained from MP Biomedicals (Irvine, CA) in a single lot (8579K). Vanadyl sulfate was obtained from Noah Technologies Corporation (San Antonio, TX) in a single lot (0210324/1.1). The manufacturer's certificate of analysis for vanadyl sulfate listed the material as vanadyl sulfate hydrate with an unspecified number of water molecules ($VOSO_4 \cdot xH_2O$) but provided the CASRN for the anhydrous form. The chemical name vanadyl sulfate and the corresponding CASRN 27774-13-6 were used for the purpose of reporting the study. Identity, purity, and stability analyses were conducted at the Battelle analytical chemistry laboratory (Columbus, OH) (Appendix A). Reports on analyses performed in support of the sodium metavanadate and vanadyl sulfate studies are on file at the National Institute of Environmental Health Sciences (NIEHS).

Lot 8579K of sodium metavanadate (a white-to-yellow powder) and lot 0210324/1.1 of vanadyl sulfate (a blue powder) were identified as sodium metavanadate and a hydrated form of vanadyl sulfate, respectively, using infrared spectroscopy and X-ray diffraction.

Moisture content was determined by weight loss on drying, Karl Fischer titration, and thermal gravimetric analysis (TGA). Karl Fischer titration was conducted by Galbraith Laboratories, Inc. (Knoxville, TN). For lot 8579K of sodium metavanadate, water content was measured at 0.2% by weight loss on drying, 0.16% by Karl Fischer titration, and 0.25% by TGA. For lot 0210324/1.1 of vanadyl sulfate, water content was measured at 21.8% by weight loss on drying, 22.8% by Karl Fischer titration, and 32.9% by TGA. The quantity of water measured by TGA was likely a more accurate estimate because it measures both crystalline and latent water, whereas weight loss on drying and Karl Fischer titration measured only adsorbed water. Overall, lot 0120324/1.1 of vanadyl sulfate was determined to be 33% water and 67% vanadyl sulfate.

Elemental analysis on both test articles was performed by scanning electron microscope energy dispersive X-ray spectroscopy, proton-induced X-ray emission (PIXE) spectroscopy, and inductively coupled plasma atomic emission spectroscopy. The PIXE analyses were conducted by Elemental Analysis, Inc. (Lexington, KY). Overall, the elemental compositions were close to the theoretical values for sodium metavanadate and vanadyl sulfate, and no major impurities were detected.

Lot 8579K of sodium metavanadate and lot 0210324/1.1 of vanadyl sulfate were analyzed using high-performance liquid chromatography (HPLC) with charged aerosol detection (CAD) (Table A-1). No impurities were detected using HPLC/CAD. The volatile content was determined by gas chromatography with flame ionization detection and electron capture detection (Table A-2). No volatiles were detected. The overall purity of both chemicals was determined to be >99%. The analytical methods and systems used for each respective lot are described in detail in Appendix A.

Accelerated stability studies confirmed the bulk chemicals were stable for at least 2 weeks when stored at temperatures of ≤60°C in sealed containers protected from light. Bulk sodium metavanadate was stored in amber plastic bottles at room temperature, and bulk vanadyl sulfate

was stored in white plastic containers at room temperature. Periodic reanalyses of the bulk chemicals were performed by the analytical chemistry laboratory before, during, and after the studies, using HPLC/CAD, and no degradation of either bulk chemical was detected.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared monthly at the Battelle study laboratory (West Jefferson, OH) by mixing sodium metavanadate or vanadyl sulfate with American Society for Testing and Materials (ASTM) Type I water, adjusted to pH 6 to 8 or pH 3.5, respectively, to give the required concentrations (Table A-3, Table A-4). Dose formulations of sodium metavanadate were prepared at six concentrations of 0, 31.3, 62.5, 125, 250, and 500 mg/L. Dose formulations of vanadyl sulfate were prepared at six concentrations of 0, 21.0, 41.9, 83.8, 168, and 335 mg/L (after being corrected for 33% water content). The formulations were prepared five times for the rat studies and three times for the mouse studies. During formulation development work, a small peak corresponding to the retention time of vanadate (V^{5+}) was observed in vanadyl sulfate formulations; the absolute amount of conversion was similar regardless of the concentration and did not increase with time.[37] The storage stability was confirmed at refrigerated and room temperatures when protected from light for up to 42 days. All dose formulations were stored at the study laboratory in plastic carboys at refrigerated temperatures (2°C–8°C) and were used within 42 days of preparation (Appendix A).

Analysis of preadministration and postadministration (animal room) dose formulations were conducted monthly at the study laboratory. All preadministration dose formulations for sodium metavanadate (Table A-5, Table A-6) were within 10% of the target concentrations. All postadministration samples were within 10% of the target concentrations, except for two animal room samples for the rat study, which were 11.7% and 10.9% below the target concentrations. All preadministration dose formulations for vanadyl sulfate (Table A-7, Table A-8) were within 10% of the target concentrations. All postadministration samples were within 10% of target concentrations, except for one animal room sample for the rat study, which was 13.7% below the target concentration.

Animal Source

Time-mated (F_0) female Sprague Dawley (Hsd:Sprague Dawley® SD®) rats were obtained from Envigo (formerly Harlan Laboratories, Inc., Haslett, MI). Male and female B6C3F1/N mice were obtained from the National Toxicology Program (NTP) colony maintained by Taconic Biosciences, Inc. (Germantown, NY).

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 and NTP animal care and use policies and applicable federal, state, and local regulations and guidelines.

Exposure Selection Rationale

Test article concentrations in drinking water for these studies were selected based on previous 14-day drinking water studies.[2; 3] The highest exposure concentrations were selected based on the magnitude of reduced water consumption, lower body weight effects, and clinical observations at concentrations higher than 500 mg/L (sodium metavanadate) and 335 mg/L (vanadyl sulfate). The test compound concentrations and the calculated vanadium concentrations are shown in Table 2.

Table 2. Concentrations of Sodium Metavanadate, Vanadyl Sulfate, and Vanadium in the Three-month Drinking Water Studies

Sodium Metavanadate		Vanadyl Sulfate	
Test Article (mg/L)	**Vanadium[a] (mg/L)**	**Test Article (mg/L)**	**Vanadium[b] (mg/L)**
0	0	0	0
31.3	13.1	21.0	6.5
62.5	26.1	41.9	13
125	52.1	83.8	26
250	104.3	168	52.1
500	208.5	335	103.9

[a]Based on vanadium composition in sodium metavanadate of 41.7%.
[b]Based on vanadium composition in vanadyl sulfate of 31%.

Three-month Studies

Study Design for Rats

F_0 female Sprague Dawley rats were 11 to 14 weeks old (sodium metavanadate) or 12 to 13 weeks old (vanadyl sulfate) upon receipt. Gestation day (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 six exposure groups for both sodium metavanadate and vanadyl sulfate on GD 5. Randomization was stratified by body weight to produce similar group mean weights using NTP Provantis software (Instem, Stone, UK).

F_0 female rats were quarantined for 12 days (sodium metavanadate) or 14 days (vanadyl sulfate) after receipt. Ten nonmated female rats 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 parasite evaluation and gross observation of disease. The health of the F_1 animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix C). All test results were negative.

Beginning on GD 6, groups of 16 F_0 female rats were administered sodium metavanadate or vanadyl sulfate in drinking water (ASTM Type I water, adjusted to a pH of approximately 7 for sodium metavanadate and 3.5 for vanadyl sulfate) throughout gestation and lactation at the following exposure concentrations: sodium metavanadate (0, 31.3, 62.5, 125, 250, or 500 mg/L) or vanadyl sulfate (0, 21.0, 41.9, 83.8, 168, or 335 mg/L). Vehicle control animals were

administered drinking water alone (ASTM Type I water, adjusted to a pH of approximately 7 for sodium metavanadate and 3.5 for vanadyl sulfate).

Feed and dosed water were available ad libitum. F_0 females were housed individually during gestation and with their respective litters during lactation. Cages were changed weekly for pregnant dams before delivery and twice weekly for dams and their litters after postnatal day (PND) 4. F_0 females were observed twice daily for signs of mortality or moribundity. Body weights were recorded upon receipt on GD 3 (vanadyl sulfate), on GD 5 (for randomization), and on GDs 6, 9, 12, 15, 18, and 21. Clinical observations were recorded daily from GD 6 through GD 21. Water consumption data were recorded on GDs 6, 9, 12, 15, 18, and 21. Details of the study design and animal maintenance are summarized in Table 3.

The day of parturition was considered lactation day (LD) 0 for dams and PND 0 for pups. F_0 females that did not deliver were euthanized on GD 27, and the uteri were examined for evidence of implantation and resorption. Body weights of littered F_0 females were recorded on LDs 1, 4, 7, 10, 13, 16, 19, 21, 25, and 28. Water consumption data were recorded on LDs 1, 4, 7, 10, 13, 16, 19, 21, 24 (sodium metavanadate only), 25, and 28. Clinical observations were recorded daily from LD 1 through LD 28. From PND 0 through PND 28, the number and sex of pups for each litter were recorded daily. On PND 1, litter weights by sex were recorded. Litter observations were recorded daily from PND 1 through PND 3, and clinical observations were recorded daily from PND 4 through PND 28. F_1 pups were individually weighed on PNDs 4, 7, 10, 13, 16, 19, 21, 25, and 28.

On PND 4, the litters were standardized to eight pups per litter (four males and four females when possible). Weaning occurred on PND 28. One male and one female from 10 litters per exposure group were randomly selected for use in the 3-month study; all available pups (n = 12 [female] or 13 [male] from four litters) were retained in the 500 mg/L sodium metavanadate study. Five pups per sex representing five litters per exposure group (except for the 500 mg/L group) were randomly selected for investigative study assessments. Two pups per sex were randomly selected from 10 litters per exposure group for an interim hematology assessment at PND 28. Five additional pups per sex (from control litters) were designated for disease monitoring. Following weaning, all F_0 females and unselected pups were humanely euthanized with carbon dioxide. Weaning marked the beginning of the 3-month study.

Groups of 10 male and 10 female F_1 pups were administered 0, 31.3, 62.5, 125, 250, or 500 mg/L sodium metavanadate or 0, 21.0, 41.9, 83.8, 168, or 335 mg/L vanadyl sulfate in drinking water for 3 months, starting on PND 28. A separate group of five male and five female F_1 pups selected for the investigative study was administered the same concentrations for internal dose assessment, urinalysis, and analysis of DNA damage (i.e., comet assay). Pups were administered the same exposure that their respective dams received during gestation and lactation. Vehicle control animals were administered drinking water alone (ASTM Type I water, adjusted to a pH of approximately 7 for sodium metavanadate or 3.5 for vanadyl sulfate).

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. F_1 rats were housed up to two per cage for males and up to four per cage for females. Cages were changed twice weekly and

rotated every 2 weeks. Details of the study design and animal maintenance are summarized in Table 3. Information on feed composition and contaminants for both diets is provided in Appendix B.

Study Design for Mice

Male and female B6C3F1/N mice were approximately 3 to 4 weeks old upon receipt. Animals were quarantined for 11 days (sodium metavanadate) or 12 days (vanadyl sulfate), and mice were approximately 6 weeks old on the first day of the study. Mice were randomly assigned to one of six exposure groups for both sodium metavanadate and vanadyl sulfate before the start of the study. Randomization was stratified by body weight to produce similar group mean weights using NTP Provantis software (Instem, Stone, UK).

Before the studies began, five male and five female mice were randomly selected for parasite evaluation, serology, and gross observation for evidence of disease. In addition, five male and five female mice were selected for 4-week and study termination serologies and parasite evaluation. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix C). All test results were negative.

Groups of 10 male and 10 female mice were administered 0, 31.3, 62.5, 125, 250, or 500 mg/L sodium metavanadate or 0, 21.0, 41.9, 83.8, 168, or 335 mg/L vanadyl sulfate in drinking water for 3 months. Vehicle control animals were administered drinking water alone (ASTM Type I water, adjusted to a pH of approximately 7 for sodium metavanadate or 3.5 for vanadyl sulfate). Male mice were housed individually, whereas female mice were housed up to five per cage. Cages were changed at least twice weekly and rotated at least every 2 weeks. Details of the study design and animal maintenance are summarized in Table 3. Information on feed composition and contaminants is given in Appendix B.

Clinical Examinations and Pathology

During the 3-month studies, rats and mice were observed twice daily for signs of morbidity or moribundity. Rats were weighed, and clinical observations and water consumption were recorded on day 1, weekly thereafter, and at study termination. Mice were weighed, and clinical observations were recorded prior to exposure on day 1, weekly thereafter, and at study termination. Water consumption by mice was reported weekly for both males and females.

Attainment of balanopreputial separation (BPS), defined as complete retraction of the prepuce from the glans penis, was evaluated in all F_1 male rats beginning on PND 35 over 21 days or until the day of attainment, and body weights were recorded upon BPS attainment. The attainment of vaginal opening (VO) was evaluated in F_1 female rats beginning on PND 25 over 18 days (sodium metavanadate) or 19 days (vanadyl sulfate) or until the day of attainment, and the corresponding body weights were recorded upon VO attainment.

At the end of the 3-month studies, urine samples were collected over a 24-hour period for urinalysis and internal dose assessment from five male and five female rats. To collect urine, animals were placed in metabolism cages and given control (0 mg/L) drinking water, and samples were collected over a 24-hour period. Urine samples collected for chemical analysis were stored frozen in a freezer set to maintain approximately −70°C until shipped to RTI International (Research Triangle Park, NC) for analysis. Urinalysis parameters were analyzed

using a Roche cobas® c311 chemistry analyzer, Roche cobas u411 urine analyzer (Roche, Indianapolis, IN), and manual methods. The parameters measured are listed in Table 3.

At the end of the 3-month rat (investigative study group) and mouse studies, the liver, duodenum, and colon (rats only) were collected, weighed, and stored frozen in a freezer set to maintain a range of −85°C to −60°C until they were shipped to Integrated Laboratory Systems, LLC (ILS, Research Triangle Park, NC) for analysis of DNA damage.

Following perinatal exposure on PND 28 (interim evaluation rats) and at the end of the 3-month studies (core study rats and mice), animals were anesthetized with a carbon dioxide/oxygen mixture and bled in random order. The blood collections and necropsies for the rat and vanadyl sulfate mouse studies were staggered over 2 consecutive days to allow adequate time for sperm motility assessments to be conducted. Blood was collected from the retroorbital plexus (rats) or retroorbital sinus (mice) for hematology, clinical chemistry (core study rats), erythrocyte micronuclei analyses (core study rats and mice), and internal dose assessment. Blood for hematology, micronuclei determinations, and internal dose assessment was collected into tubes containing tripotassium ethylenediaminetetraacetic acid (K_3 EDTA). Blood for clinical chemistry was collected into serum separator tubes and centrifuged, and the serum was harvested. Hematology parameters were analyzed using an Advia® 120 hematology analyzer (Bayer Diagnostics Division, Tarrytown, NY). Clinical chemistry parameters were analyzed using a Roche cobas c311 chemistry analyzer (Roche, Indianapolis, IN). The parameters measured are listed in Table 3. Samples for erythrocyte micronuclei determination were stored at 2°C–8°C immediately after collection and shipped to Integrated Laboratory Systems, LLC (ILS, Research Triangle Park, NC) for analysis. For excretion and internal dose assessment, following 24 hours in metabolism cages for urine collection, plasma was collected within 2 hours of lights-on, and all samples were collected within 2.5 hours (Table 3). Plasma was isolated from a fraction of whole blood, and both samples were stored at −60°C to −85°C until they were shipped to RTI International for chemical analysis (Research Triangle Park, NC).

Samples were collected for sperm motility and vaginal cytology evaluations from F_1 male and female rats and male and female mice in the 0, 125, 250, and 500 mg/L sodium metavanadate groups and in the 0, 83.8, 168, and 335 mg/L vanadyl sulfate groups. For 16 consecutive days before scheduled study termination, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were collected and subsequently stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for sperm count and motility. An incision was made in the distal region of the left cauda epididymis, and the cauda was placed in a Petri dish containing M199 solution (maintained at approximately 37°C) with 1.0% bovine serum albumin (BSA). A small sample of the diluted sperm was loaded into a 100 µm-chambered slide for determination of motility. After completion of sperm motility estimates, the remainder of the diluted sperm and cauda epididymis in M199/BSA solution and the left testis were stored frozen (approximately −70°C) until enumeration of sperm concentration was performed. The left cauda epididymis in M199/BSA solution was thawed and homogenized, and the left testis was thawed and homogenized in 0.9% saline with 0.05% Triton-X 100. Homogenized samples were mixed with a DNA-specific fluorescent dye (IDENT) to allow for sperm identification under fluorescent illumination.

Necropsies were performed on all F_1 rats in the core study and all mice. Organ weights were determined for the left and right epididymis, heart, left and right kidney, liver, lungs, left and right ovary, left and right testis, and thymus. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin (except eyes, which were first fixed in Davidson's solution, and testis, vaginal tunics, and epididymis, 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. Complete histopathological examinations were performed by the study laboratory pathologist on all F_1 rats and mice to a no-effect level. Table 3 lists the tissues and organs examined.

The laboratory report and select histopathology slides were reviewed by quality assessment (QA) pathologists, who also served as coordinators of the Pathology Working Group (PWG). The QA report and the reviewed slides were submitted to the Division of Translational Toxicology (DTT) pathologist, who reviewed and addressed any inconsistencies in the diagnoses made by the study laboratory and QA pathologists. The QA/PWG pathologist 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. 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[38] and Boorman et al.[39]

Table 3. Experimental Design and Materials and Methods in the Three-month Studies of Sodium Metavanadate and Vanadyl Sulfate

Rats	Mice
Study Laboratory	
Battelle (Columbus, OH)	Same as in rats
Strain and Species	
Sprague Dawley (Hsd:Sprague Dawley® SD®)	B6C3F1/N
Animal Source	
Envigo (formerly Harlan Laboratories, Inc., Haslett, MI)	Taconic Biosciences, Inc. (Germantown, NY)
Time Held Before Studies	
Sodium metavanadate: 4 days Vanadyl sulfate: 4 days	Sodium metavanadate: 11 days Vanadyl sulfate: 12 days
Average Age When Studies Began	
F_0 females (sodium metavanadate): 11–15 weeks F_0 females (vanadyl sulfate): 12–14 weeks	6 weeks
Date of First Exposure	
F_0 females (sodium metavanadate): February 12, 2016 F_0 females (vanadyl sulfate): May 1, 2016 F_1 rats (sodium metavanadate): March 27–28, 2016 F_1 rats (vanadyl sulfate): June 13–15, 2016	Sodium metavanadate: January 25 (females) or 26 (males), 2016 Vanadyl sulfate: April 11 (females) or 12 (males), 2016

Rats	Mice
Duration of Exposure	
F_0 females: GD 6 through LD 28 F_1 rats: Perinatal plus 3 months	3 months
Date of Last Exposure	
F_0 females (sodium metavanadate): March 27–28, 2016 F_0 females (vanadyl sulfate): June 13–15, 2016 F_1 rats (sodium metavanadate): June 27–29, 2016 F_1 rats (vanadyl sulfate): September 12–16, 2016	Sodium metavanadate: April 25 (females) or 26 (males), 2016 Vanadyl sulfate: July 11 (females) or 13 (males), 2016
Necropsy Dates	
F_1 rats (sodium metavanadate): June 27–29, 2016 F_1 rats (vanadyl sulfate): September 14–16, 2016	Sodium metavanadate: April 25–26, 2016 Vanadyl sulfate: July 11–13, 2016
Average Age at Necropsy	
F_1 rats: 17 weeks	19 weeks
Size of Study Groups	
F_0 females: 16/group F_1 rats (interim evaluation): 20/sex/group F_1 rats (core study): 10/sex/group F_1 rats (investigative study): 5/sex/group	10/sex/group
Method of Distribution	
Animals were distributed randomly into groups of approximately equal initial mean body weights using NTP Provantis (Instem, Stone, UK).	Same as in rats
Animals per Cage	
F_0 females: 1 (with litter) F_1 rats: up to 2 (males) or up to 4 (females)	1 (males) or up to 5 (females)
Method of Animal Identification	
F_0 females: Cage card and tail marking	Cage card and tail tattoo
F_1 rats: Limb tattoo until PND 28, then cage card and tail mark/tattoo	
Diet	
F_0 females: Irradiated NIH-07 wafer feed (Zeigler Brothers, Gardners, PA), available ad libitum, changed at least weekly	Irradiated NTP-2000 wafer feed (Zeigler Brothers, Gardners, PA), available ad libitum, changed at least weekly
F_1 rats: Irradiated NTP-2000 wafer feed (Zeigler Brothers, Gardners, PA), available ad libitum, changed at least weekly	
Water	
ASTM Type I water (adjusted to a pH of ~7 for sodium metavanadate and 3.5 for vanadyl sulfate), either untreated or containing a formulation of sodium metavanadate or vanadyl sulfate via water bottles, available ad libitum	Same as in rats

Rats	Mice
Cages	
Solid polycarbonate (Lab Products, Inc., Seaford, DE), changed at least weekly (individually housed F_0 females and their litters through PND 4) or twice weekly (group-housed rats). After PND 28, cage rotation occurred at least every 2 weeks.	Solid polycarbonate (Lab Products, Inc., Seaford, DE), changed at least weekly and rotated at least every 2 weeks
Bedding	
Irradiated Sani-Chips® (P.J. Murphy Forest Products Corporation, Montville, NJ), changed with cage changes	Same as in rats
Racks	
Stainless steel (Lab Products, Inc., Seaford, DE), changed and rotated every 2 weeks	Same as in rats
Rack Filters	
Spun-bonded polyester (National Filter Media Corporation, Olive Branch, MS), changed every 2 weeks	Same as in rats
Animal Room Environment	
Temperature: 70°F–73°F Relative humidity: 28%–65% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour	Temperature: 67°F–74°F Relative humidity: 28%–60% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour
Exposure Concentrations	
Sodium metavanadate: 0, 31.3, 62.5, 125, 250, or 500 mg/L in drinking water	Same as in rats
Vanadyl sulfate: 0, 21.0, 41.9, 83.8, 168, or 335 mg/L in drinking water	
Type and Frequency of Observation	
F_0 females: Observed twice daily. Weighed on GDs 3 (vanadyl sulfate), 5, 6, 9, 12, 15, 18, and 21 and on LDs 1, 4, 7, 10, 13, 16, 19, 21, 25, and 28. Water consumption was measured on GDs 6, 9, 12, 15, 18, and 21 and on LDs 1, 4, 7, 10, 13, 16, 19, 21, 24 (sodium metavanadate), 25, and 28. Clinical observations were recorded daily from GD 6 through LD 28.	Observed twice daily. Weighed at randomization, prior to exposure on day 1, weekly thereafter, and at study termination. Clinical observations were recorded weekly. Water consumption was reported weekly.
F_1 rats: Observed twice daily. Litter weights by sex were recorded on PND 1. The number and sex of pups were recorded daily throughout the lactation period. Pups were weighed individually on PNDs 4, 7, 10, 13, 16, 19, 21, 25, and 28, weekly thereafter, and at study termination. Clinical observations were recorded from PND 4 through PND 28, weekly thereafter, and at study termination. Water consumption was recorded weekly. Vaginal opening (and concomitant body weight) was evaluated beginning on PND 25, and balanopreputial separation (and concomitant body weight) was evaluated beginning on PND 35.	
Method of Euthanasia	
F_0 females and F_1 internal dose assessment rats: carbon dioxide and/or exsanguination	Carbon dioxide

Rats	Mice
F_1 core study rats: carbon dioxide	
Necropsy	
F_1 core study rats: Complete necropsies were performed on all animals. Organs weighed at study termination were left and right epididymis, heart, left and right kidney, liver, lungs, left and right ovary, left and right testis, and thymus.	Same as in rats
Clinical Pathology	
Following perinatal exposure on PND 28 (interim evaluation rats) and at study termination (core study rats), blood was collected from the retroorbital plexus for hematology, clinical chemistry (core study rats), and erythrocyte micronuclei (core study rats).	At study termination, blood was collected from the retroorbital sinus for hematology and erythrocyte micronuclei.
Hematology: hematocrit; manual hematocrit; hemoglobin concentration; red blood cell, nucleated red blood cell, reticulocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; white blood cell count and differential; morphological evaluation	Same as in rats
Clinical chemistry: urea nitrogen, creatinine, glucose, total protein, albumin, globulin, A/G ratio, cholesterol, triglycerides, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, total bile acids	None
Histopathology	
F_1 core study: Complete histopathology was performed to a no-effect level, plus on all animals in the control groups. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal glands, brain (seven sections including [1] olfactory bulbs, [2] fronto-parietal cortex and basal ganglia, [3] mid-parietal cortex and thalamus, [4] mid-brain with substantia nigra and red nucleus, [5] posterior colliculi, [6] mid-cerebellum including cranial nerve VIII, and [7] posterior medulla), cervix/vagina, clitoral glands, esophagus, eyes, femur (including diaphysis with marrow cavity and epiphysis [femoral condyle with epiphyseal cartilage plate, articular cartilage, and articular surface]), Harderian glands, heart and aorta, kidneys, large intestine (cecum, colon, rectum), liver (two sections including left lateral lobe and median lobe), lungs and mainstem bronchi, lymph nodes (mandibular and mesenteric), mammary gland with adjacent (inguinal) skin, muscle (thigh), nasal cavity and nasal turbinates, nerve (sciatic, tibial, and trigeminal with ganglion), ovaries, pancreas, parathyroid glands, pituitary gland, preputial glands, prostate, salivary glands, seminal vesicle, small intestine (duodenum, jejunum, ileum), spinal cord, spleen, stomach (forestomach and glandular), testis with epididymis,	Complete histopathology was performed to a no-effect level, plus on all animals in the control groups. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, brain (seven sections including [1] olfactory bulbs, [2] fronto-parietal cortex and basal ganglia, [3] mid-parietal cortex and thalamus, [4] mid-brain with substantia nigra and red nucleus, [5] posterior colliculi, [6] mid-cerebellum including cranial nerve VIII, and [7] posterior medulla), cervix/vagina, clitoral glands, esophagus, eyes, femur (including diaphysis with marrow cavity and epiphysis [femoral condyle with epiphyseal cartilage plate, articular cartilage, and articular surface]), gallbladder, Harderian glands, heart and aorta, kidneys, large intestine (cecum, colon, rectum), liver (two sections including left lateral lobe and median lobe), lungs and mainstem bronchi, lymph nodes (mandibular and mesenteric), mammary gland, muscle (thigh) (sodium metavanadate), nasal cavity and nasal turbinates, nerve (sciatic and trigeminal with ganglion), ovaries, pancreas, parathyroid glands, pituitary glands, preputial glands, prostate gland, salivary glands, seminal vesicle, small intestine (duodenum, jejunum, ileum), spinal cord, spleen, stomach (forestomach and glandular), testis with epididymis, thymus, thyroid gland, trachea, urinary bladder, and uterus.

Rats	Mice
thymus, thyroid gland, trachea, urinary bladder, and uterus.	
Sperm Motility and Vaginal Cytology	
F_1 core study rats: At study termination, sperm samples were collected from male rats in the 0, 125, 250, and 500 mg/L (sodium metavanadate) groups and in the 0, 83.8, 168, and 335 mg/L (vanadyl sulfate) groups for sperm count and sperm motility evaluations. The cauda epididymis was weighed. Vaginal samples were collected for up to 16 consecutive days before study termination from female rats in the 0, 125, 250, and 500 mg/L (sodium metavanadate) groups and in the 0, 83.8, 168, and 335 mg/L (vanadyl sulfate) groups for vaginal cytology evaluations.	Same as in rats
F_1 Investigative Study	
Excretion and internal dose assessment: At study termination, urine and plasma were collected for chemical analysis. For urine collection, animals were placed in metabolism cages and given control (0 mg/L) drinking water, and urine was collected over a 24-hour period. Plasma was then collected within 2 hours of lights-on, and all samples were collected within 2.5 hours. Results of the plasma and urine analyses have been reported previously.[40]	None
Urinalysis: At study termination, urine samples were collected as described above and analyzed for appearance, volume, specific gravity, microscopic assessment, total protein, glucose, creatinine, calcium, phosphorus, N-acetyl-β-glucosaminidase, alkaline phosphatase, and aspartate aminotransferase.	None
Comet assay: At study termination, samples of liver, duodenum, and colon epithelium were collected for the comet assay. Due to sample quality and analysis issues, comet assay data were not analyzed or reported.	At study termination, samples of liver and duodenum were collected for the comet assay. Due to sample quality and analysis issues, comet assay data were not analyzed or reported.

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

Statistical Methods

Statistical methods were chosen based on distributional assumptions as well as on the need to incorporate within-litter correlation among animals (for the perinatal rat studies). Unless specifically mentioned, all endpoints were tested for a trend across exposure groups, followed by pairwise tests for each exposed group against the control group. Significance of all trend and pairwise tests is reported at both 0.05 and 0.01 levels.

Calculation and Analysis of Gross and Nonneoplastic Lesion Incidences

For mice and rats, incidences of gross findings and histopathology were summarized as number of animals affected. Fisher's exact test,[41] a procedure that uses the overall proportion of affected animals, was used to determine statistical significance between exposed and vehicle control animals, and the Cochran-Armitage trend test was used to test for significant trends.[42]

Analysis of Continuous Variables

Before statistical analysis, outliers identified using the Dixon and Massey test[43] for small samples ($n < 20$) and Tukey's outer fences method[44] for large samples ($n \geq 20$) were examined by DTT personnel, and biologically implausible values (likely due to experimental error) were eliminated from the analysis.

In most instances, no considerations for litter effects were necessary in the analysis of the continuous data. This was the case for all of the mice data, for the F_0 rat data, and for F_1 rat data in which the cohort consisted of only one animal per litter. In these instances, organ and body weight measurements, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett[45] and Williams.[46; 47]

When litter effects were present, body weight endpoints were analyzed using linear mixed models, with litters as a random effect. To adjust for multiple comparisons, a Dunnett-Hsu adjustment was used.[48] Pup weights were adjusted for litter size, as described below.

Water consumption, clinical chemistry, hematology, urinalysis, tissue concentration, litter sizes, gestational length, pup survival, spermatid, and epididymal spermatozoal measurements typically have skewed distributions. When litter effects were not present, these endpoints were analyzed using the nonparametric multiple comparison methods of Shirley[49] (as modified by Williams[50]) and Dunn.[51] For all continuous variables without litter effects, the Jonckheere test[52] 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).

When litter effects were present for non-normally distributed continuous endpoints (e.g., hematology data for F_1 interim rats), 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[53]). Pairwise comparisons were made using a modified Wilcoxon test that incorporated litter effects.[54] The Hommel procedure was used to adjust for multiple comparisons.[55]

For the sodium metavanadate F_1 core study rats, only the 500 mg/L group contained multiple animals per sex per litter. Because all other groups in the study contained only one animal per litter, litter-based methods were not used in the analysis of these animals.

Analysis of Gestational and Fertility Indices

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

Body Weight Adjustments

Because body weights typically decrease with increasing litter size, adjusting body weight for litter size in the analysis of fetal and pup weights can provide additional precision to detect test article effects.[56] Body weight adjustments are appropriate when the litter effect, as evidenced by

decreasing weights with increasing litter size, is relatively constant across exposure concentrations. Preweaning pup body weights were adjusted for live litter size by fitting a linear model to body weights as a function of exposure concentration and litter size, with the coefficient of litter size retained for adjustment as above. 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.

Analysis of Time-to-event Data

Time-to-event endpoints, such as day of attainment of BPS and VO, have several features that require careful model selection: non-normality of distributions, litter-based correlation, and censored values when attainment was not observed before the end of the observation period. Further, growth retardation, reflected in the weaning weight, is an important covariate in the case of BPS and VO given the relationship between normal day of expected attainment and body weight.

When attainment times were approximately normally distributed and attainment was observed for all or most animals, two approaches for modeling discrete developmental endpoints were taken. First, a mixed model was fit to attainment day as a function of exposure concentration with a random litter effect. For BPS and VO, a second mixed model was fit to attainment day as a function of exposure concentration and weaning weight with a random litter effect. Dunnett-Hsu adjustments were used to account for multiple comparisons.[48]

To calculate mean attainment values adjusted for weaning weight, a linear model was fit to attainment day as a function of exposure concentration and weaning weight. The estimated coefficient of weaning weight was then used to adjust each attainment day based on the difference between the measured weaning weight and the mean weaning weight.

Analysis of Vaginal Cytology Data

Vaginal cytology data consist of daily observations of estrous cycle stages over a 16-day period. Differences from the control group for cycle length and the number of cycles were analyzed using the Shirley and Dunn tests, as described above.

To identify disruptions in estrous cyclicity, a continuous-time Markov chain model (multistate model) was fit using a maximum likelihood approach,[57] producing estimates of stage lengths for each exposure group. Confidence intervals for these estimates were obtained from bootstrap sampling of the individual animal cycle sequences. Stage lengths that were significantly different from the control animals were identified using permutation testing.

Quality Assurance Methods

The 3-month studies were conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice Regulations.[58] In addition, the 3-month 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 Toxicity Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed

and assessed by DTT staff, and all comments were resolved or otherwise addressed during the preparation of this toxicity report.

Genetic Toxicology

The genetic toxicity of sodium metavanadate and vanadyl sulfate was assessed by testing whether the chemicals induced mutations in various strains of *Salmonella typhimurium* and *Escherichia coli* and increased the frequency of micronucleated erythrocytes in rat and mouse peripheral blood. The protocol for these studies and the results are given in Appendix D.

The genetic toxicity studies have evolved from an earlier effort to develop a comprehensive database permitting a critical anticipation of a chemical's carcinogenicity in experimental animals based on numerous considerations, including the relationship between the molecular structure of the chemical and its observed effects in short-term in vitro and in vivo genetic toxicity tests (structure-activity relationships). The short-term tests were developed originally to clarify proposed mechanisms of chemical-induced DNA damage given the relationship between electrophilicity and mutagenicity[59] and the somatic mutation theory of cancer.[60; 61] Not all cancers, however, 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.[62] 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).[63; 64] Additionally, no battery of tests that included the *Salmonella* test improved predictivity over the *Salmonella* test alone. Other tests, however, can provide useful information on the types of DNA and chromosomal damage induced by the chemical under investigation.

Peripheral Blood Micronucleus Test

Micronuclei (literally "small nuclei" or Howell-Jolly bodies) 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.[65; 66] Acute in vivo bone marrow chromosome aberration and micronucleus tests appear to be less predictive of carcinogenicity than the *Salmonella* test.[67; 68] 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.[69] Because of the theoretical and observed associations between induced genetic damage and adverse effects in somatic and germ cells, determination of in vivo genetic effects is important to overall understanding of risks associated with exposure to a particular chemical.

Results

Data Availability

All study data were evaluated. 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-TOX-106.[70]

Sodium Metavanadate

Rats

Three-month Study (Perinatal Phase)

Around the time of parturition and shortly thereafter (approximately gestation day [GD] 22 to lactation day [LD] 4), adverse clinical signs (e.g., lethargy, hunched posture, and pallor) and exposure-related mortalities were observed in dams in the 250 and 500 mg/L groups (Appendix E). Higher dam mortality was also observed in later lactation (LD 5–25) in the 500 mg/L group. Due to early deaths, moribund euthanasia, and whole litter losses, nine dams and their respective litters in the 500 mg/L group were removed during late gestation or early lactation (Table 4). Three additional dams were removed in early lactation due to insufficient litter size. These removals collectively resulted in only four dams (and litters) at the end of lactation in the 500 mg/L group available to populate the postweaning study. Three dams in the 250 mg/L group were removed because of moribundity, and an additional dam in the 250 mg/L group was found dead. Two dams in the 125 mg/L group were removed from the study in early lactation due to moribundity and whole litter loss.

Table 4. Summary of the Disposition of F_0 Female Rats during Perinatal Exposure in the Perinatal and Three-month Drinking Water Study of Sodium Metavanadate

Reproductive Performance	0 mg/L	31.3 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L
Time-mated Females (GD 6)	16	16	16	16	16	16
Females Pregnant (%)[a]	16 (100)	14 (87.5)	16 (100)	16 (100)	15 (93.8)	16 (100)
Females Not Pregnant (%)	0 (0)	2 (12.5)	0 (0)	0 (0)	1 (6.25)	0 (0)
Pregnant Females Removed Prior to Littering (%)[b]	0 (0)	0 (0)	0 (0)	0 (0)	2 (13.3)	1 (6.25)
Dams with Litters on LD 0 (%)[b]	16 (100)	14 (100)	16 (100)	16 (100)	13 (100)	15 (100)
Gestation Length (Days)[c,d]	22.1 ± 0.1 (16)	22.3 ± 0.2 (14)	22.0 ± 0.0 (16)	22.2 ± 0.1 (16)	22.0 ± 0.1 (13)	22.5 ± 0.2 (15)
Litters Poststandardization (PND 4)[e]	15	12	14	14	11	8[f]
Litters at Weaning (PND 28)	15	12	14	14	11	4[g]
F_1 Males/Females (PND 28)[h]	57/63	44/52	52/59	56/55	45/35	13/12

GD = gestation day; LD = lactation day; PND = postnatal day.
[a]Statistical analysis performed by the Cochran-Armitage (trend) and Fisher's exact (pairwise) tests.
[b]Percentage is given as a portion of pregnant dams.
[c]Statistical analysis performed by the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.
[d]Gestation length calculated for sperm-positive females that delivered a litter. Data are presented as mean ± standard error (number of dams).
[e]Standardization to eight pups/litter (four pups/sex).
[f]There were seven exposure-related dam and respective litter removals between LD 0 and LD 4 (poststandardization): two dams euthanized moribund on LD 0, one whole litter loss on PND 1 and PND 4, and three removed during standardization on LD 4 due to insufficient litter sizes (less than four total pups) resulting from high pup mortality (PND 0–4).
[g]There were four exposure-related dam and respective litter removals between LD 4 (poststandardization) and LD 25: one dam found dead on LD 5 and one on LD 20 and one dam euthanized moribund on LD 7 and one on LD 25.
[h]Number of males/females surviving until weaning on PND 28.

Beginning on GD 12, dams in the 250 and 500 mg/L groups had significantly decreased body weights compared to that of the control group, and on GD 21, significantly decreased body weights were observed in exposed groups ≥125 mg/L, with a 14% decrease at 500 mg/L (Table 5). Decreased body weights persisted during lactation in the 250 and 500 mg/L groups until LDs 21 and 25, respectively, after which the body weights were no longer different from those of the control group, indicating recovery.

Table 5. Summary of 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 Metavanadate

Parameter[a,b]	0 mg/L	31.3 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L
Gestation Body Weight						
Gestation Day						
6	242.1 ± 2.4 (16)	237.8 ± 3.1 (14)	240.7 ± 3.2 (16)	240.3 ± 3.1 (16)	240.9 ± 3.6 (15)	239.2 ± 3.1 (16)
9	253.5 ± 2.9* (16)	249.7 ± 3.8 (14)	250.6 ± 3.3 (16)	248.1 ± 3.3 (16)	245.8 ± 3.0 (15)	243.9 ± 2.5 (16)
12	272.2 ± 2.9** (16)	269.7 ± 3.6 (14)	266.3 ± 3.5 (16)	261.4 ± 3.3* (16)	257.1 ± 2.9** (15)	256.2 ± 2.4** (16)

Parameter[a,b]	0 mg/L	31.3 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L
15	290.7 ± 3.0** (16)	286.5 ± 3.7 (14)	286.0 ± 3.8 (16)	281.1 ± 4.0 (16)	275.6 ± 3.2** (15)	271.9 ± 2.8** (16)
18	333.8 ± 3.8** (16)	325.3 ± 5.5 (14)	326.5 ± 5.2 (16)	320.8 ± 5.6 (16)	308.7 ± 4.8** (15)	307.7 ± 4.0** (16)
21	385.0 ± 4.0** (16)	368.2 ± 7.9 (14)	373.3 ± 7.2 (16)	360.0 ± 8.6* (16)	348.9 ± 5.3** (14)	330.8 ± 6.6** (16)
Gestation Weight Change						
Gestation Day Interval						
6–9	11.4 ± 1.1** (16)	11.8 ± 2.5 (14)	9.9 ± 0.7 (16)	7.8 ± 1.6 (16)	4.9 ± 1.6** (15)	4.8 ± 1.2** (16)
9–12	18.7 ± 0.7** (16)	20.0 ± 1.0 (14)	15.6 ± 0.8* (16)	13.4 ± 1.0** (16)	11.3 ± 0.8** (15)	12.3 ± 1.0** (16)
12–15	18.5 ± 0.6 (16)	16.8 ± 1.4 (14)	19.8 ± 1.3 (16)	19.7 ± 1.6 (16)	18.5 ± 1.1 (15)	15.7 ± 1.5 (16)
15–18	43.1 ± 1.3** (16)	38.8 ± 2.8 (14)	40.5 ± 2.9 (16)	39.7 ± 2.5 (16)	33.1 ± 4.3* (15)	35.8 ± 2.3* (16)
18–21	51.2 ± 1.1** (16)	42.9 ± 3.6 (14)	46.8 ± 2.8 (16)	39.3 ± 4.4* (16)	37.3 ± 3.9* (14)	23.1 ± 5.7** (16)
6–21	142.9 ± 2.5** (16)	130.4 ± 7.3 (14)	132.6 ± 6.7 (16)	119.7 ± 7.9* (16)	109.5 ± 5.9** (14)	91.6 ± 7.5** (16)
Lactation Body Weight						
Lactation Day						
1	282.9 ± 3.1** (16)	280.1 ± 4.0 (14)	275.4 ± 4.5 (16)	270.2 ± 4.4 (15)	263.1 ± 3.3** (11)	243.8 ± 7.5** (12)
4	297.6 ± 3.2** (16)	290.4 ± 3.8 (14)	292.1 ± 4.8 (16)	280.4 ± 5.0 (14)	268.7 ± 3.9** (11)	220.2 ± 13.6** (12)
7	305.5 ± 3.9** (15)	303.9 ± 4.2 (12)	300.1 ± 5.3 (14)	288.5 ± 4.1* (14)	279.8 ± 4.5** (11)	250.6 ± 14.1** (7)
10	314.0 ± 4.3** (15)	314.4 ± 4.5 (12)	309.4 ± 5.4 (14)	303.0 ± 4.9 (14)	293.4 ± 3.8** (11)	278.9 ± 6.2** (6)
13	319.2 ± 3.6** (15)	316.4 ± 4.2 (12)	311.2 ± 4.6 (14)	312.0 ± 5.4 (14)	295.3 ± 2.8** (11)	279.2 ± 4.6** (6)
16	314.3 ± 4.8** (15)	320.4 ± 4.5 (12)	314.1 ± 6.9 (14)	308.9 ± 4.0 (14)	297.5 ± 4.1* (11)	290.0 ± 7.8** (6)
19	311.9 ± 5.1** (15)	316.2 ± 5.1 (12)	309.3 ± 7.1 (14)	308.1 ± 4.3 (14)	299.5 ± 5.4 (11)	284.7 ± 11.3** (6)
21	307.3 ± 3.1** (15)	311.2 ± 5.2 (12)	304.6 ± 6.2 (14)	300.6 ± 3.8 (14)	287.0 ± 4.4** (11)	279.1 ± 11.0** (5)
25	286.4 ± 3.8* (15)	289.8 ± 4.2 (12)	283.2 ± 5.8 (14)	281.7 ± 4.7 (14)	278.1 ± 3.5 (11)	252.7 ± 23.4** (5)
28	284.8 ± 4.1 (15)	287.3 ± 5.4 (12)	277.4 ± 5.9 (14)	278.5 ± 4.7 (14)	275.3 ± 4.6 (11)	278.8 ± 6.2 (4)

Parameter[a,b]	0 mg/L	31.3 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L
Lactation Weight Change						
Lactation Day Interval						
1–4	14.7 ± 2.2** (16)	10.3 ± 2.4 (14)	16.7 ± 2.1 (16)	9.0 ± 2.8 (14)	5.6 ± 2.0 (11)	−23.6 ± 8.0** (12)
4–7	7.0 ± 1.7 (15)	11.9 ± 2.4 (12)	6.5 ± 1.8 (14)	8.2 ± 3.0 (14)	11.1 ± 2.3 (11)	−1.0 ± 7.4 (7)
7–10	8.5 ± 2.3* (15)	10.4 ± 1.7 (12)	9.3 ± 2.5 (14)	14.5 ± 3.7 (14)	13.6 ± 2.3 (11)	14.6 ± 4.7 (6)
10–13	5.2 ± 3.2 (15)	2.0 ± 1.3 (12)	1.7 ± 2.7 (14)	9.0 ±2.3 (14)	1.8 ± 2.6 (11)	0.3 ± 2.8 (6)
13–16	−4.9 ± 3.2 (15)	4.1 ± 2.4 (12)	3.0 ± 3.6 (14)	−3.1 ± 2.0 (14)	2.2 ± 2.4 (11)	10.8 ± 3.5** (6)
16–19	−2.4 ± 2.7 (15)	−4.3 ± 2.4 (12)	−4.8 ± 1.9 (14)	−0.8 ± 2.0 (14)	2.0 ± 3.1 (11)	−5.3 ± 15.7 (6)
19–21	−4.6 ± 3.1 (15)	−5.0 ± 2.1 (12)	−4.7 ± 3.0 (14)	−7.5 ± 2.4 (14)	−12.6 ± 3.8 (11)	−13.7 ± 4.7 (5)
21–25	−20.9 ± 2.5* (15)	−21.4 ± 2.0 (12)	−21.4 ± 2.2 (14)	−18.9 ± 3.4 (14)	−8.9 ± 3.6 (11)	−26.4 ± 15.2 (5)
25–28	−1.6 ± 1.4 (15)	−2.5 ± 2.4 (12)	−5.8 ± 1.9 (14)	−3.2 ± 2.1 (14)	−2.7 ± 1.9 (11)	3.3 ± 6.6 (4)
4–28	−13.7 ± 3.4** (15)	−4.8 ± 3.1 (12)	−16.3 ± 2.2 (14)	−1.9 ± 3.2* (14)	6.6 ± 4.8** (11)	23.2 ± 2.3** (4)
1–28	0.6 ± 3.2** (15)	5.3 ± 2.9 (12)	1.1 ± 3.2 (14)	7.1 ± 3.5 (14)	12.2 ± 3.8* (11)	18.9 ± 4.4** (4)

Statistical significance for an exposed 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 exposed 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 are presented as mean ± standard error (number of dams). Body weight data are presented in grams.

Water consumption (g water/kg body weight/day) was significantly decreased during gestation (GD 6–21) in groups ≥125 mg/L in a concentration-dependent manner, reaching a 37% decrease at 500 mg/L (Table 6). Water consumption began to increase in these groups during lactation, with an average 7% decrease in the 500 mg/L group compared to the control group from LD 1 to LD 13. Water consumption by groups ≤250 mg/L was similar to the control group during lactation. Sodium metavanadate intake (mg sodium metavanadate/kg body weight/day) during gestation (GD 6–21) and lactation (LD 1–13) are presented in Table 6. Due to reduced water consumption during gestation, sodium metavanadate intake was less than dose-proportional (10-fold increase in intake versus a 16-fold increase in drinking water concentration from the 31.3 mg/L to 500 mg/L groups).

Table 6. Summary of Water and Sodium Metavanadate Consumption by F_0 Female Rats during Gestation and Lactation in the Perinatal and Three-month Drinking Water Study

Parameter[a]	0 mg/L	31.3 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L
Gestation Day Interval (g/kg/day)[b]						
6–9	132.4 ± 3.9** (16)	131.2 ± 6.0 (14)	129.2 ± 3.1 (16)	112.7 ± 2.6** (16)	96.4 ± 2.9** (15)	79.7 ± 2.9** (16)
9–12	134.5 ± 4.1** (16)	138.5 ± 6.0 (14)	137.0 ± 4.2 (16)	119.7 ± 4.2* (15)	102.9 ± 5.6** (15)	82.8 ± 2.2** (16)
12–15	133.8 ± 3.8** (16)	135.3 ± 5.8 (13)	130.5 ± 4.5 (16)	120.0 ± 6.2* (16)	101.0 ± 4.8** (15)	79.9 ± 2.9** (16)
15–18	145.4 ± 3.6** (16)	143.8 ± 6.5 (14)	142.7 ± 4.7 (16)	129.7 ± 5.4* (16)	116.2 ± 5.2** (15)	101.6 ± 2.6** (15)
18–21	133.8 ± 3.8** (16)	134.7 ± 8.1 (14)	128.6 ± 4.2 (16)	118.5 ± 5.4* (16)	99.8 ± 7.4** (15)	83.5 ± 4.7** (16)
6–21	134.6 ± 3.4** (16)	135.5 ± 6.1 (14)	132.1 ± 3.5 (16)	119.6 ± 4.4* (16)	102.7 ± 4.2** (15)	84.7 ± 2.6** (16)
Lactation Day Interval (g/kg/day)[b]						
1–4	169.0 ± 11.2 (16)	166.5 ± 6.5 (13)	184.1 ± 9.1 (16)	196.6 ± 7.8 (14)	172.0 ± 9.5 (11)	107.4 ± 16.4* (12)
4–7	197.1 ± 5.6 (15)	197.2 ± 6.0 (12)	206.0 ± 4.3 (14)	197.3 ± 7.2 (14)	191.9 ± 11.1 (11)	156.0 ± 27.1 (7)
7–10	224.1 ± 6.9 (14)	228.5 ± 3.9 (12)	232.9 ± 5.3 (14)	227.3 ± 6.7 (14)	211.3 ± 13.9 (11)	236.2 ± 13.6 (6)
10–13	265.5 ± 9.8** (15)	250.7 ± 6.2 (12)	257.3 ± 8.0 (14)	240.0 ± 4.3 (14)	221.6 ± 11.7 (11)	228.0 ± 22.3 (6)
1–13	215.0 ± 6.7 (15)	213.3 ± 4.3 (12)	224.3 ± 4.8 (14)	215.9 ± 5.1 (14)	200.0 ± 10.7 (11)	199.1 ± 15.4 (6)
Chemical Intake (mg/kg/day)[c,d]						
GD 6–21	0.0 ± 0.0 (16)	4.2 ± 0.2 (14)	8.3 ± 0.2 (16)	14.9 ± 0.6 (16)	25.7 ± 1.0 (15)	42.4 ± 1.3 (16)
LD 1–13	0.0 ± 0.0 (15)	6.7 ± 0.1 (12)	14.0 ± 0.3 (14)	27.0 ± 0.6 (14)	50.0 ± 2.7 (11)	99.5 ± 7.7 (6)

Statistical significance for an exposed 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 are presented as mean ± standard error (number of dams).
[b]Each exposed 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.
[c]Chemical intake calculated as: ([exposure concentration × water consumption]/[average body weight of day range]).
[d]No statistical analysis was performed on the chemical intake data.

Sodium metavanadate exposure had no effect on pregnancy or littering rate. While not reaching statistical significance, the total number and number of live pups per litter on postnatal day (PND) 0 in the 500 mg/L group were lower than control rats by 19% and 25%, respectively (Table 7). The total number and number of live pups per litter on PND 0 in the 250 mg/L group were also slightly lower but within 10% of the control group. Pup viability after PND 4 was lower in the 250 and 500 mg/L groups. The number of live pups per litter was significantly

decreased relative to the control group, beginning poststandardization on PND 4 in the 250 and 500 mg/L groups, and there were higher numbers of dead pups per litter and lower survival ratios at all postnatal intervals (PND 0, PND 1–4, PND 5–28) in the 500 mg/L group. Adverse clinical observations in pups in the 500 mg/L group included absence of milk band, pallor, thinness, ruffled coat, and cold to touch (Appendix E). Similar findings were observed in the 250 mg/L group but at a lower incidence.

Table 7. Summary of 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 Metavanadate

Postnatal Day	0 mg/L	31.3 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L
Total Litter Size[a,b]						
0	13 ± 0.4 (16)	11.1 ± 1.1 (14)	12.6 ± 1.0 (16)	12.9 ± 1.0 (16)	11.8 ± 0.9 (13)	10.5 ± 0.6 (15)
Live Litter Size[a,b]						
0	12.3 ± 0.5 (16)	10.9 ± 1.1 (14)	12.4 ± 1.0 (16)	12.9 ± 1.0 (15)[c]	11.4 ± 1.0 (11)[c]	9.2 ± 1.0 (12)[c]
1	12.3 ± 0.6 (16)	10.6 ± 1.1 (14)	12.2 ± 1.0 (16)	12.7 ± 1.0 (15)	11.3 ± 1.1 (11)	8.3 ± 1.2 (12)
4 (Prestandardization)	12.3 ± 0.6 (16)	10.5 ± 1.1 (14)	12.2 ± 1.0 (16)	13.4 ± 0.6 (14)[c]	11.1 ± 1.0 (11)	7.4 ± 1.4 (11)[c]
4 (Poststandardization)	8.0 ± 0.0** (15)[c]	8.0 ± 0.0 (12)[c]	8.0 ± 0.0 (14)[c]	8.0 ± 0.0 (14)	7.5 ± 0.4** (11)	6.8 ± 0.5** (8)[c]
7	8.0 ± 0.0** (15)	8.0 ± 0.0 (12)	8.0 ± 0.0 (14)	7.9 ± 0.1 (14)	7.5 ± 0.4* (11)	7.0 ± 0.7** (6)[d]
10	8.0 ± 0.0** (15)	8.0 ± 0.0 (12)	7.9 ± 0.1 (14)	7.9 ± 0.1 (14)	7.5 ± 0.4 (11)	7.0 ± 0.7* (6)
13	8.0 ± 0.0** (15)	8.0 ± 0.0 (12)	7.9 ± 0.1 (14)	7.9 ± 0.1 (14)	7.4 ± 0.4** (11)	7.0 ± 0.7* (6)
16	8.0 ± 0.0** (15)	8.0 ± 0.0 (12)	7.9 ± 0.1 (14)	7.9 ± 0.1 (14)	7.4 ± 0.4** (11)	7.0 ± 0.7* (6)
19	8.0 ± 0.0** (15)	8.0 ± 0.0 (12)	7.9 ± 0.1 (14)	7.9 ± 0.1 (14)	7.3 ± 0.4** (11)	7.0 ± 0.7* (6)
21	8.0 ± 0.0** (15)	8.0 ± 0.0 (12)	7.9 ± 0.1 (14)	7.9 ± 0.1 (14)	7.3 ± 0.4** (11)	6.4 ± 0.9** (5)[d]
25	8.0 ± 0.0** (15)	8.0 ± 0.0 (12)	7.9 ± 0.1 (14)	7.9 ± 0.1 (14)	7.3 ± 0.4** (11)	5.8 ± 1.0** (5)
28	8.0 ± 0.0** (15)	8.0 ± 0.0 (12)	7.9 ± 0.1 (14)	7.9 ± 0.1 (14)	7.3 ± 0.4** (11)	6.3 ± 1.1** (4)[d]
% Live Male per Litter[a,b,e]						
0	41.59 ± 3.04** (16)	50.62 ± 5.06 (14)	47.18 ± 3.87 (16)	53.68 ± 4.91* (15)	62.28 ± 3.42** (11)	54.84 ± 5.67** (12)
No. of Dead Pups (Litters)[f,g]						
0	11 (3)	3 (2)	3 (2)	13 (5)	29 (3)	47 (7)
1–4	1 (1)	5 (3)	3 (3)	7 (7)	3 (3)	29 (5)
5–28	0 (0)	0 (0)	1 (1)	1 (1)	2 (2)	9 (4)

Postnatal Day	0 mg/L	31.3 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L
Dead per Litter[a,b,h]						
0	0.69 ± 0.44* (16)	0.21 ± 0.15 (14)	0.19 ± 0.14 (16)	0.81 ± 0.50 (16)	2.23 ± 1.45 (13)	3.13 ± 1.17 (15)
1–4	0.06 ± 0.06** (16)	0.36 ± 0.23 (14)	0.19 ± 0.10 (16)	0.47 ± 0.13 (15)	0.27 ± 0.14 (11)	2.42 ± 1.08* (12)
5–28	0.00 ± 0.00** (15)	0.00 ± 0.00 (12)	0.07 ± 0.07 (14)	0.07 ± 0.07 (14)	0.18 ± 0.12 (11)	1.80 ± 1.07** (5)
Survival Ratio[a,b]						
0[i]	0.95 ± 0.03* (16)	0.98 ± 0.01 (14)	0.99 ± 0.01 (16)	0.91 ± 0.06 (16)	0.84 ± 0.10 (13)	0.70 ± 0.11 (15)
1–4[j]	0.99 ± 0.01** (16)	0.97 ± 0.02 (14)	0.96 ± 0.03 (16)	0.90 ± 0.07 (15)	0.97 ± 0.02 (11)	0.73 ± 0.10* (12)
5–28[k]	1.00 ± 0.00** (15)	1.00 ± 0.00 (12)	0.99 ± 0.01 (14)	0.99 ± 0.01 (14)	0.98 ± 0.02 (11)	0.70 ± 0.18** (5)

Statistical significance for an exposed 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 are presented as mean of the litter means ± standard error (number of litters).
[b]Each exposed 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.
[c]There were 16 dam and respective litter removals between lactation day (LD) 0 and LD 4 (poststandardization). One dam in the control group and two in both the 31.3 mg/L and 62.5 mg/L groups were removed during standardization on LD 4 due to insufficient litter sizes (less than four total pups). In the 125 mg/L group, one dam was euthanized moribund on LD 0, and one whole litter loss occurred on LD 3. In the 250 mg/L group, one whole litter loss occurred on LD 0 and the respective dam was euthanized on LD 1, and one dam was euthanized moribund on LD 0. In the 500 mg/L group, one whole litter loss occurred on LD 0 and the respective dam was euthanized on LD 1, two dams were euthanized moribund on LD 0, one whole litter loss occurred on LD 1 and one on LD 4, and three dams and their respective litters were removed during standardization on LD 4 due to insufficient litter sizes resulting from high pup mortality (LD 0–4).
[d]There were four dam and respective litter removals between LD 4 (poststandardization) and LD 25. In the 500 mg/L group, one dam was found dead on LD 5 and one on LD 20, and one dam was euthanized moribund on LD 7 and one on LD 25.
[e]100 × [number of live males in exposure group]/[number of live males and females in exposure group](number of pups).
[f]Total number of dead pups in exposure group (number of litters contributing dead pups).
[g]No statistical analysis was performed on this endpoint.
[h]Number dead per litter (number of litters).
[i]Survival per litter: Number of live pups on postnatal day (PND) 0/total number of pups upon completion of parturition.
[j]Survival per litter: Number of pups prestandardization on PND 4/total live pups on PND 0.
[k]Survival per litter: Number of live pups on PND 28/number of live pups poststandardization on PND 4.

Pup body weights at the end of lactation were within 10% of those of the control groups in all exposed groups except the 500 mg/L groups, in which male body weight decreased by 21% and female body weight decreased by 22% compared to the respective control groups (Table 8). Other exposed groups exhibited sporadically significant differences from the control groups throughout lactation but were generally within 10% of the control groups.

Table 8. Summary of Preweaning F_1 Male and Female Rat Pup Body Weights Following Perinatal Exposure to Sodium Metavanadate

Postnatal Day	0 mg/L	31.3 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L
Male						
1[a,b,c]	7.54 ± 0.07* (81/16)	7.57 ± 0.19 (68/14)	7.57 ± 0.12 (93/16)	7.27 ± 0.16 (98/15)	7.57 ± 0.16 (76/11)	6.83 ± 0.27* (55/12)
4[d,e,f,g]	10.91 ± 0.11** (81/16)	10.83 ± 0.29 (67/14)	10.70 ± 0.21 (93/16)	10.88 ± 0.25 (95/14)	10.67 ± 0.18 (76/11)	8.34 ± 0.79** (39/9)
7[d,e,h]	17.70 ± 0.28** (57/15)	17.45 ± 0.52 (44/12)	17.49 ± 0.48 (52/14)	16.71 ± 0.44 (56/14)	16.93 ± 0.39 (46/11)	13.90 ± 1.17** (21/7)
10[d,e,h]	25.25 ± 0.42** (57/15)	24.97 ± 0.60 (44/12)	25.36 ± 0.54 (52/14)	23.88 ± 0.49 (56/14)	23.22 ± 0.40* (46/11)	21.16 ± 0.87** (19/6)
13[d,e,h]	33.12 ± 0.50** (57/15)	32.57 ± 0.59 (44/12)	33.05 ± 0.56 (52/14)	30.76 ± 0.48** (56/14)	29.61 ± 0.46** (46/11)	26.44 ± 1.13** (19/6)
16[d,e,h]	40.50 ± 0.59** (57/15)	39.96 ± 0.70 (44/12)	39.75 ± 0.65 (52/14)	37.33 ± 0.56** (56/14)	35.44 ± 0.67** (46/11)	31.31 ± 1.15** (19/6)
19[d,e,h]	51.27 ± 0.68** (57/15)	50.39 ± 1.33 (44/12)	50.23 ± 0.98 (52/14)	47.20 ± 0.88** (56/14)	45.19 ± 0.69** (45/11)	38.66 ± 1.38** (19/6)
21[d,e,h]	60.96 ± 0.61** (57/15)	59.73 ± 1.43 (44/12)	60.00 ± 1.03 (52/14)	56.03 ± 0.92** (56/14)	53.63 ± 0.86** (45/11)	45.84 ± 1.64** (15/5)
25[d,e,h]	79.18 ± 0.85** (57/15)	79.56 ± 1.58 (44/12)	78.69 ± 1.38 (52/14)	75.14 ± 1.03 (56/14)	73.80 ± 0.99* (45/11)	63.29 ± 2.27** (14/5)
28[d,e,h]	97.60 ± 1.28** (57/15)	98.73 ± 1.98 (44/12)	97.31 ± 1.72 (52/14)	91.62 ± 1.34* (56/14)	90.94 ± 1.16* (45/11)	77.34 ± 3.18** (13/4)
Female						
1	7.13 ± 0.09 (115/16)	7.20 ± 0.14 (80/13)	7.25 ± 0.08 (102/15)	7.07 ± 0.11 (93/14)	7.00 ± 0.14 (48/11)	6.53 ± 0.33* (45/11)
4	10.18 ± 0.17** (115/16)	10.20 ± 0.24 (80/13)	10.37 ± 0.13 (102/15)	10.39 ± 0.22 (92/14)	10.18 ± 0.14 (46/11)	8.37 ± 0.87** (37/8)
7	16.55 ± 0.33** (63/15)	16.67 ± 0.41 (52/12)	16.79 ± 0.39 (60/14)	16.17 ± 0.38 (55/14)	16.14 ± 0.30 (36/11)	14.12 ± 0.71** (23/6)
10	23.72 ± 0.41** (63/15)	24.01 ± 0.43 (52/12)	24.53 ± 0.44 (59/14)	23.35 ± 0.43 (55/14)	21.92 ± 0.29* (36/11)	20.23 ± 0.72** (23/6)
13	31.30 ± 0.53** (63/15)	31.58 ± 0.39 (52/12)	32.15 ± 0.49 (59/14)	29.96 ± 0.50 (55/14)	28.33 ± 0.33** (35/11)	25.54 ± 0.94** (23/6)
16	38.24 ± 0.62** (63/15)	38.51 ± 0.42 (52/12)	38.47 ± 0.55 (59/14)	36.30 ± 0.58 (55/14)	34.18 ± 0.44** (35/11)	30.33 ± 1.00** (23/6)
19	47.85 ± 0.77** (63/15)	48.17 ± 0.83 (52/12)	47.68 ± 0.83 (59/14)	45.81 ± 0.84 (55/14)	43.34 ± 0.62** (35/11)	36.65 ± 1.77** (23/6)
21	56.34 ± 0.78** (63/15)	56.17 ± 0.97 (52/12)	56.59 ± 0.82 (59/14)	54.05 ± 0.82 (55/14)	50.35 ± 0.80** (35/11)	45.51 ± 1.41** (17/5)
25	71.80 ± 0.63** (63/15)	72.67 ± 1.24 (52/12)	72.11 ± 0.97 (59/14)	70.01 ± 0.93 (55/14)	67.90 ± 0.91* (35/11)	58.87 ± 3.62** (16/5)
28	85.99 ± 0.89** (63/15)	87.68 ± 1.50 (52/12)	87.47 ± 1.23 (59/14)	83.91 ± 1.19 (55/14)	82.38 ± 1.58 (35/11)	66.99 ± 5.91** (12/4)

Postnatal Day	0 mg/L	31.3 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L
Male and Female						
1	7.33 ± 0.08* (196/16)	7.42 ± 0.16 (148/14)	7.42 ± 0.09 (195/16)	7.12 ± 0.14 (191/15)	7.34 ± 0.13 (124/11)	6.69 ± 0.28* (100/12)
4	10.52 ± 0.14** (196/16)	10.57 ± 0.27 (147/14)	10.49 ± 0.16 (195/16)	10.62 ± 0.22 (187/14)	10.47 ± 0.16 (122/11)	8.20 ± 0.80** (76/9)
7	17.09 ± 0.28** (120/15)	17.04 ± 0.44 (96/12)	17.09 ± 0.41 (112/14)	16.42 ± 0.39 (111/14)	16.59 ± 0.35 (82/11)	13.48 ± 1.14** (44/7)
10	24.45 ± 0.38** (120/15)	24.47 ± 0.47 (96/12)	24.87 ± 0.47 (111/14)	23.59 ± 0.44 (111/14)	22.67 ± 0.37* (82/11)	20.61 ± 0.75** (42/6)
13	32.17 ± 0.49** (120/15)	32.05 ± 0.44 (96/12)	32.53 ± 0.49 (111/14)	30.37 ± 0.45* (111/14)	29.07 ± 0.40** (81/11)	25.91 ± 0.99** (42/6)
16	39.33 ± 0.58** (120/15)	39.19 ± 0.50 (96/12)	39.02 ± 0.56 (111/14)	36.82 ± 0.52** (111/14)	34.93 ± 0.54** (81/11)	30.78 ± 1.05** (42/6)
19	49.49 ± 0.65** (120/15)	49.21 ± 1.03 (96/12)	48.84 ± 0.84 (111/14)	46.48 ± 0.79* (111/14)	44.37 ± 0.65** (80/11)	37.65 ± 1.45** (42/6)
21	58.55 ± 0.61** (120/15)	57.80 ± 1.14 (96/12)	58.11 ± 0.85 (111/14)	55.01 ± 0.78* (111/14)	52.23 ± 0.81** (80/11)	45.57 ± 1.42** (32/5)
25	75.32 ± 0.54** (120/15)	75.80 ± 1.32 (96/12)	75.14 ± 1.08 (111/14)	72.56 ± 0.86 (111/14)	71.25 ± 0.94* (80/11)	59.75 ± 3.22** (30/5)
28	91.54 ± 0.92** (120/15)	92.78 ± 1.69 (96/12)	92.06 ± 1.37 (111/14)	87.76 ± 1.07 (111/14)	87.02 ± 1.25 (80/11)	73.24 ± 2.94** (25/4)

Statistical significance for an exposed 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 are presented as mean ± standard error (number of pups/number of litters). Body weight data are presented in grams.
[b]Each exposed 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]Total pup weight on postnatal day (PND) 1 divided by number of live pups on PND 1.
[d]Data are presented as mean of the litter means ± standard error (number of pups/number of litters). Body weight data are presented in grams.
[e]Statistical analysis performed using mixed models with random litter effect for both trend and pairwise tests, using the Dunnett-Hsu adjustment for multiple comparisons.
[f]PND 4 prestandardization.
[g]Individual pup weights first adjusted for live litter size on PND 1.
[h]Individual pup weights first adjusted for live litter size on PND 4 poststandardization.

Three-month Study (Postweaning Phase)

During the 3-month postweaning phase, one male and two females in the 500 mg/L group were euthanized moribund with clinical observations of ruffled coat, hunched, and lethargy (Appendix E). All other animals survived until scheduled removal. Male and female rats in exposed groups ≤250 mg/L had body weights within 10% of the respective control groups, with sporadic but significant decreases in males and increases in females compared to the control groups. Body weights of the 500 mg/L group were significantly decreased relative to those of the control group for males and females throughout the postweaning period. Terminal body weight at 500 mg/L was significantly decreased (by 27% for males and 11% for females) compared to that of control animals (Table 9, Table 10; Figure 1).

Table 9. Summary of Survival and Body Weights of Male Rats in the Perinatal and Three-month Drinking Water Study of Sodium Metavanadate

Postnatal Day	0 mg/L		31.3 mg/L			62.5 mg/L			125 mg/L			250 mg/L			500 mg/L		
	Wt. (g)[a,b]	n[c]	Wt. (g)	Wt. (% of Controls)	n	Wt. (g)	Wt. (% of Controls)	n	Wt. (g)	Wt. (% of Controls)	n	Wt. (g)	Wt. (% of Controls)	n	Wt. (g)	Wt. (% of Controls)	n
28[d]	96.9 ± 1.7**	15 (12)	99.3 ± 2.1	102.4	15 (11)	98.4 ± 1.9	101.5	15 (12)	92.5 ± 1.6	95.4	15 (11)	92.2 ± 1.7	95.1	15 (10)	79.0 ± 3.7**	81.4	13 (4)
35	134.0 ± 2.3**	15 (12)	137.0 ± 2.6	102.2	15 (11)	138.1 ± 2.9	103.1	15 (12)	128.2 ± 1.9	95.7	15 (11)	127.1 ± 2.5	94.9	15 (10)	99.2 ± 4.6**	74.0	12 (4)
42	181.9 ± 2.5**	15 (12)	183.6 ± 3.3	100.9	15 (11)	186.5 ± 3.5	102.6	15 (12)	174.1 ± 3.1	95.7	15 (11)	169.6 ± 3.2	93.3	15 (10)	131.7 ± 7.8**	72.4	12 (4)
49	231.6 ± 3.4**	15 (12)	235.7 ± 3.7	101.8	15 (11)	237.5 ± 4.3	102.5	15 (12)	219.0 ± 3.4	94.6	15 (11)	216.6 ± 3.8	93.5	15 (10)	170.0 ± 12.4**	73.4	12 (4)
56	280.2 ± 3.8**	15 (12)	283.7 ± 4.0	101.2	15 (11)	283.4 ± 4.5	101.1	15 (12)	261.8 ± 4.0*	93.4	15 (11)	259.4 ± 3.6*	92.5	15 (10)	208.1 ± 15.2**	74.2	12 (4)
63	320.3 ± 4.5**	15 (12)	325.2 ± 3.9	101.5	15 (11)	322.8 ± 6.6	100.8	15 (12)	301.5 ± 4.7	94.1	15 (11)	295.5 ± 4.5*	92.2	15 (10)	239.9 ± 17.3**	74.9	12 (4)
70	353.6 ± 5.3**	15 (12)	353.4 ± 5.0	99.9	15 (11)	353.7 ± 5.6	100.0	15 (12)	328.6 ± 5.1*	92.9	15 (11)	321.6 ± 5.0**	91.0	15 (10)	265.4 ± 18.2**	75.1	12 (4)
77	377.8 ± 6.0**	15 (12)	378.2 ± 5.8	100.1	15 (11)	377.6 ± 5.8	100.0	15 (12)	352.3 ± 5.1*	93.3	15 (11)	345.4 ± 5.9**	91.4	15 (10)	285.1 ± 18.0**	75.5	12 (4)
84	397.8 ± 6.3**	15 (12)	399.0 ± 5.6	100.3	15 (11)	402.0 ± 6.3	101.1	15 (12)	370.2 ± 5.6*	93.1	15 (11)	364.4 ± 5.9**	91.6	15 (10)	301.2 ± 17.7**	75.7	12 (4)
91	415.3 ± 6.3**	15 (12)	419.0 ± 6.0	100.9	15 (11)	418.2 ± 6.1	100.7	15 (12)	388.0 ± 5.8*	93.4	15 (11)	382.6 ± 7.2**	92.1	15 (10)	315.4 ± 16.3**	75.9	12 (4)
98	427.2 ± 6.1**	15 (12)	429.2 ± 6.7	100.5	15 (11)	424.2 ± 5.9	99.3	15 (12)	398.5 ± 6.9*	93.3	15 (11)	392.0 ± 7.3**	91.8	15 (10)	322.0 ± 16.7**	75.4	12 (4)
105	441.8 ± 6.1**	15 (12)	440.0 ± 6.9	99.6	15 (11)	434.4 ± 6.9	98.3	15 (12)	407.4 ± 7.0**	92.2	15 (11)	399.1 ± 7.7**	90.3	15 (10)	329.5 ± 18.5**	74.6	12 (4)
112	453.0 ± 6.6**	15 (12)	449.6 ± 7.4	99.2	15 (11)	449.2 ± 7.0	99.2	15 (12)	416.5 ± 7.4**	91.9	15 (11)	407.3 ± 7.9**	89.9	15 (10)	338.4 ± 16.9**	74.7	12 (4)
119[e,f]	459.1 ± 7.4**	10[f]	452.9 ± 9.0	98.6	10	460.7 ± 8.0	100.4	10	424.1 ± 10.1*	92.4	10	413.3 ± 9.2**	90.0	10	335.3 ± 10.0**	73.0	12

Statistical significance for an exposed 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]Statistical analysis performed using mixed models with random litter effect for both trend and pairwise tests, using the Dunnett-Hsu adjustment for multiple comparisons.

[b]Weights shown are mean of the litter means ± standard error.

[c]Number of individual animals (number of litters); includes F_1 core and investigative study animals.

[d]Postnatal day (PND) 28 is the day animals were placed on study after pups were weaned.

[e]Statistical analysis performed by the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.

[f]Number of individual animals includes only the F_1 core animals. Investigative study animals were removed on PND 116 (females) and PND 117 (males).

Table 10. Summary of Survival and Body Weights of Female Rats in the Perinatal and Three-month Drinking Water Study of Sodium Metavanadate

Postnatal Day	0 mg/L		31.3 mg/L			62.5 mg/L			125 mg/L			250 mg/L			500 mg/L		
	Wt. (g)[a,b]	n[c]	Wt. (g)	Wt. (% of Controls)	n	Wt. (g)	Wt. (% of Controls)	n	Wt. (g)	Wt. (% of Controls)	n	Wt. (g)	Wt. (% of Controls)	n	Wt. (g)	Wt. (% of Controls)	n
28[d]	85.3 ± 1.3**	15 (12)	89.7 ± 1.4	105.2	15 (11)	88.3 ± 1.1	103.6	15 (12)	83.1 ± 1.6	97.4	15 (11)	84.4 ± 1.2	99.0	15 (10)	66.1 ± 6.6**	77.5	12 (4)
35	109.8 ± 3.7**	15 (12)	114.9 ± 2.1	104.6	15 (11)	116.9 ± 1.7	106.4	15 (12)	110.4 ± 2.6	100.6	15 (11)	109.4 ± 1.7	99.7	15 (10)	88.2 ± 3.6**	80.4	11 (3)
42	143.8 ± 2.4**	15 (12)	148.1 ± 2.8	103.0	15 (11)	147.7 ± 2.2	102.7	15 (12)	142.6 ± 3.3	99.1	15 (11)	139.5 ± 2.6	97.0	15 (10)	105.7 ± 7.6**	73.5	11 (3)
49	170.4 ± 2.4**	15 (12)	174.9 ± 3.0	102.6	15 (11)	171.3 ± 2.1	100.5	15 (12)	167.5 ± 3.4	98.3	15 (11)	166.3 ± 3.0	97.6	15 (10)	132.6 ± 5.2**	77.8	10 (3)
56	191.6 ± 2.5**	15 (12)	199.1 ± 3.1	103.9	15 (11)	193.7 ± 2.4	101.1	15 (12)	190.5 ± 4.5	99.4	15 (11)	189.9 ± 2.9	99.1	15 (10)	156.2 ± 5.3**	81.5	10 (3)
63	208.5 ± 2.6**	15 (12)	218.6 ± 3.8	104.8	15 (11)	210.4 ± 2.6	100.9	15 (12)	206.6 ± 4.9	99.1	15 (11)	211.2 ± 3.4	101.3	15 (10)	177.7 ± 3.6**	85.2	10 (3)
70	219.7 ± 4.0**	15 (12)	233.9 ± 4.4*	106.5	15 (11)	225.6 ± 3.2	102.7	15 (12)	219.6 ± 5.5	100.0	15 (11)	225.4 ± 3.5	102.6	15 (10)	190.7 ± 2.6**	86.8	10 (3)
77	229.4 ± 3.6**	15 (12)	248.8 ± 4.0*	108.5	14 (10)[e]	233.7 ± 3.3	101.9	15 (12)	229.1 ± 6.0	99.8	15 (11)	237.4 ± 3.2	103.5	15 (10)	202.8 ± 2.4**	88.4	10 (3)
84	238.1 ± 3.7**	15 (12)	257.7 ± 5.1*	108.3	15 (11)	241.3 ± 3.6	101.3	15 (12)	239.5 ± 6.7	100.6	15 (11)	244.1 ± 3.3	102.5	15 (10)	208.3 ± 1.6**	87.5	10 (3)
91	244.7 ± 3.6*	15 (12)	267.1 ± 4.0**	109.2	15 (11)	254.2 ± 4.5	103.9	15 (12)	250.2 ± 6.6	102.2	15 (11)	258.2 ± 3.5	105.5	15 (10)	222.6 ± 2.4*	91.0	10 (3)
98	251.7 ± 3.3**	15 (12)	267.8 ± 3.2*	106.4	14 (11)[e]	253.4 ± 3.4	100.7	15 (12)	251.0 ± 6.3	99.7	15 (11)	259.0 ± 3.7	102.9	15 (10)	223.2 ± 2.4**	88.7	10 (3)
105	256.5 ± 3.7**	15 (12)	275.8 ± 4.5*	107.5	15 (11)	257.0 ± 4.4	100.2	15 (12)	256.1 ± 6.7	99.9	15 (11)	261.6 ± 3.3	102.0	15 (10)	225.6 ± 2.3**	87.9	10 (3)
112	261.5 ± 3.0**	15 (12)	279.2 ± 5.3*	106.8	15 (11)	263.3 ± 3.8	100.7	15 (12)	259.6 ± 6.5	99.3	15 (11)	266.2 ± 3.2	101.8	15 (10)	230.4 ± 2.8**	88.1	10 (3)
119[f,g]	265.7 ± 1.9**	10[g]	282.7 ± 5.7	106.4	10	267.5 ± 4.9	100.7	10	266.7 ± 8.7	100.4	10	272.5 ± 4.6	102.6	10	235.1 ± 3.1**	88.5	10

Statistical significance for an exposed 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]Statistical analysis performed using mixed models with random litter effect for both trend and pairwise tests, using the Dunnett-Hsu adjustment for multiple comparisons.

[b]Weights shown are mean of the litter means ± standard error.

[c]Number of individual animals (number of litters); includes F_1 core and investigative study animals.

[d]Postnatal day (PND) 28 is the day animals were placed on study after pups were weaned.

[e]One F_1 core female PND 77 body weight from the 31.3 mg/L group and one F_1 investigative study female PND 98 body weight from the 31.3 mg/L group were excluded as outliers.

[f]Statistical analysis performed by the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.

[g]Number of individual animals includes only the F_1 core animals. Investigative study animals were removed on PND 116 (females) and PND 117 (males).

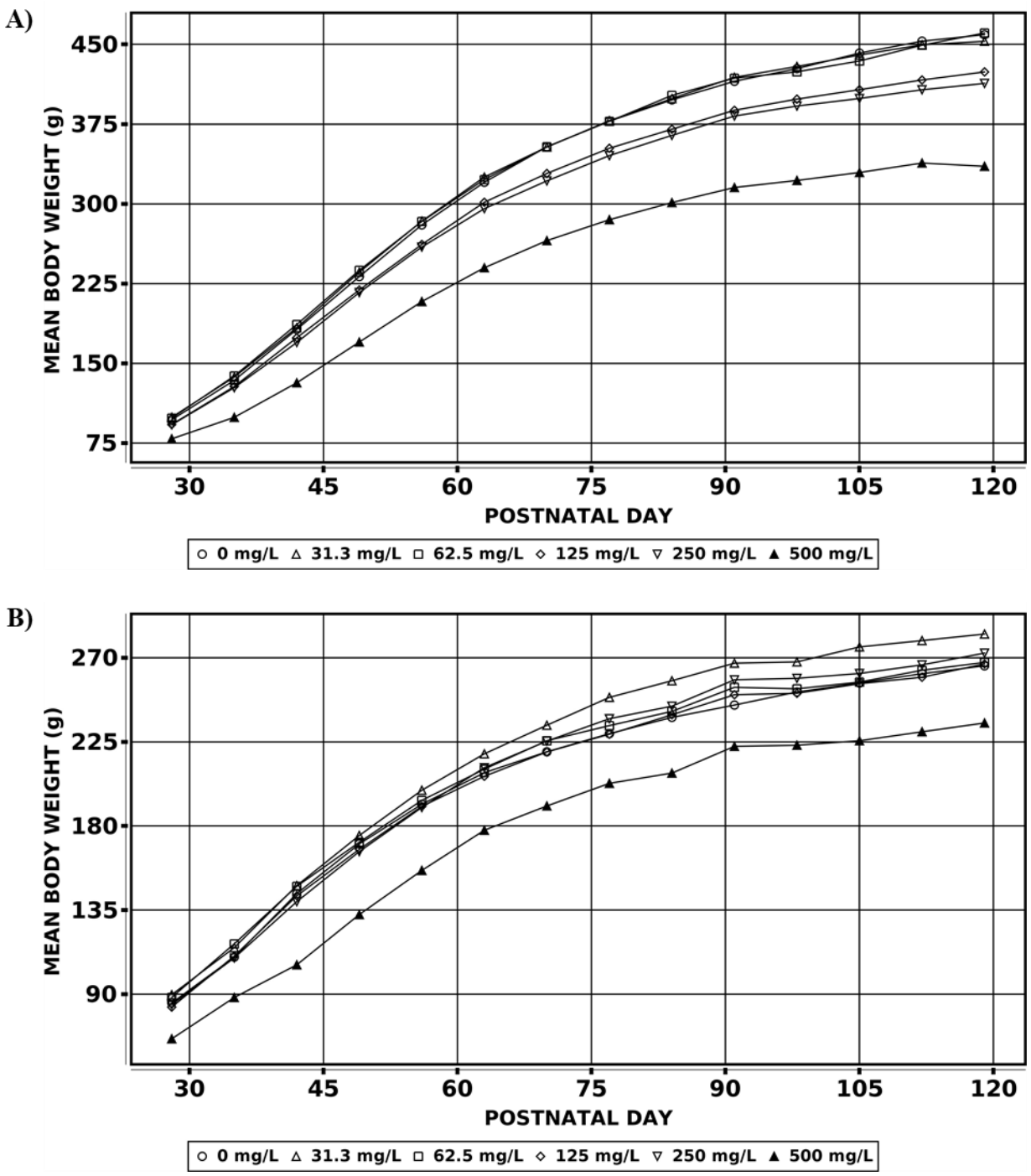


Figure 1. Growth Curves for Male and Female Rats in the Perinatal and Three-month Drinking Water Study of Sodium Metavanadate

Growth curves are shown for (A) males and (B) females.

Water consumption continued to be lower in a concentration-related fashion throughout the postweaning period (PND 28–112). Water consumption over the PND 28–112 interval by males exposed to 250 and 500 mg/L was significantly decreased by 11% and 16%, respectively, compared to that of the control group (Table 11). Water consumption by exposed females was lower in comparison to the control group over the PND 28–112 interval, with a significant decrease of 19% and 28% at 250 and 500 mg/L, respectively. For both males and females, consumption at concentrations ≤125 mg/L was similar to that of the control groups throughout the postweaning period. Sodium metavanadate intake during the 3-month postweaning phase (PND 28–112) is presented in Table 11. Due to reduced water consumption over the course of the study, sodium metavanadate intake was less than dose-proportional (12- to 13-fold increase in intake by males and females versus 16-fold increase in drinking water concentration for the 31.3 mg/L to 500 mg/L groups).

Table 11. Summary of Water and Sodium Metavanadate Consumption by Male and Female Rats in the Perinatal and Three-month Drinking Water Study

Postnatal Day[a]	0 mg/L	31.3 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L
Male (g/kg/day)[b]						
28–35	147.0 ± 3.9* (8)	146.2 ± 4.9 (9)	161.0 ± 4.1 (7)	159.1 ± 7.6 (9)	143.3 ± 2.6 (8)	111.5 ± 6.1* (7)
49–56	121.6 ± 3.1** (8)	128.0 ± 2.7 (9)	122.0 ± 4.3 (8)	122.4 ± 4.7 (9)	109.4 ± 3.2* (8)	101.7 ± 2.6** (7)
70–77	79.9 ± 2.5** (8)	80.0 ± 1.2 (9)	78.0 ± 2.0 (8)	80.5 ± 2.7 (9)	71.7 ± 2.4 (8)	71.8 ± 2.5 (7)
105–112	57.2 ± 2.1** (8)	62.3 ± 1.4 (9)	59.4 ± 1.6 (8)	55.6 ± 1.5 (9)	49.5 ± 1.2 (8)	47.8 ± 1.3* (7)
28–112	86.4 ± 2.1** (8)	89.3 ± 1.3 (9)	88.2 ± 1.5 (8)	87.1 ± 2.6 (9)	76.9 ± 1.8* (8)	72.4 ± 1.3** (7)
Chemical Intake (mg/kg/day)[c,d]						
28–112	0.0 ± 0.0 (8)	2.8 ± 0.0 (9)	5.5 ± 0.1 (8)	10.9 ± 0.3 (9)	19.2 ± 0.5 (8)	36.2 ± 0.6 (7)
Female (g/kg/day)						
28–35	133.9 ± 14.8 (5)	151.2 ± 13.8 (6)	151.7 ± 4.4 (5)	149.8 ± 7.3 (8)	142.2 ± 2.5 (5)	119.5 ± 8.5 (4)
49–56	126.8 ± 3.2** (5)	120.7 ± 10.6 (6)	123.3 ± 6.4 (5)	118.6 ± 1.5 (8)	106.3 ± 3.3** (5)	101.6 ± 7.2** (4)
70–77	94.8 ± 3.7** (5)	92.3 ± 7.9 (6)	95.7 ± 3.7 (5)	92.8 ± 2.9 (8)	79.2 ± 3.3* (5)	72.6 ± 4.9* (4)
105–112	85.6 ± 5.9** (5)	78.8 ± 5.2 (6)	78.4 ± 2.7 (5)	79.6 ± 2.4 (8)	61.3 ± 3.1** (5)	54.7 ± 3.3** (4)
28–112	105.5 ± 1.6** (5)	101.3 ± 7.2 (6)	103.5 ± 3.3 (5)	101.7 ± 2.3 (8)	85.4 ± 3.4** (5)	75.8 ± 4.4** (4)
Chemical Intake (mg/kg/day)						
28–112	0.0 ± 0.0 (5)	3.2 ± 0.2 (6)	6.5 ± 0.2 (5)	12.7 ± 0.3 (8)	21.4 ± 0.9 (5)	37.9 ± 2.2 (4)

Statistical significance for an exposed 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 are presented as mean ± standard error (number of cages).
[b]Each exposed 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.
[c]Chemical intake calculated as: ([exposure concentration × water consumption]/[average body weight of day range]).
[d]No statistical analysis was performed on the chemical intake data.

Exposure to sodium metavanadate did not affect the time to balanopreputial separation (BPS) attainment (Appendix E). Vaginal opening (VO) was significantly delayed by 3.2 days in the 250 mg/L group (Table 12). The significant delay was also observed when adjusted for body weight on day of attainment. Furthermore, body weight at attainment in the 250 mg/L group was significantly increased relative to the control group. Pubertal markers were not evaluated at 500 mg/L due to the magnitude of body weight effects; therefore, 250 mg/L was the highest exposure concentration available for VO analysis.

Table 12. Summary of Vaginal Opening of F_1 Female Rats Exposed to Sodium Metavanadate in the Perinatal and Three-month Drinking Water Study

Parameter[a]	0 mg/L	31.3 mg/L	62.5 mg/L	125 mg/L	250 mg/L
No. Examined[b]	15 (12)	15 (11)	15 (12)	15 (11)	15 (10)
No. Not Attaining[c]	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)
Day of VO					
Litter mean[d,e]	36.8 ± 0.9**	38.1 ± 0.9	37.0 ± 0.5	37.7 ± 0.7	40.0 ± 0.6**
Adjusted litter mean[d,f,g]	36.7 ± 0.9**	38.4 ± 0.9	37.2 ± 0.5	37.4 ± 0.7	39.9 ± 0.6**
Body Weight at Attainment (g)[h]	120.2 ± 3.9	127.3 ± 3.3	124.7 ± 2.7	122.0 ± 4.1	129.8 ± 3.0*
Body Weight at Weaning (g)[h]	85.3 ± 1.2*	89.3 ± 1.5	88.6 ± 1.1	82.8 ± 1.5	84.6 ± 1.3

Statistical significance for an exposed 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 $p \leq 0.05$; **$p \leq 0.01$.
VO = vaginal opening.
[a]Data are displayed as mean ± standard error unless otherwise noted; values are based on litter means, not individual pup values.
[b]No. Examined = the number of pups examined (number of litters).
[c]No. Not Attaining = number of pups (number of litters) that survived to the end of the observation period without attaining VO.
[d]Summary statistics and mixed model results are presented for animals that attained during the observation period.
[e]Statistical analysis performed using mixed effects models with exposure concentration as a covariate, litter as a random effect for both trend and pairwise tests, and a Dunnett-Hsu adjustment for multiple pairwise comparisons.
[f]Statistical analysis performed using mixed effects models with exposure concentration and weaning weight as covariates, litter as a random effect for both trend and pairwise tests, and a Dunnett-Hsu adjustment for multiple pairwise comparisons.
[g]Adjusted based on body weight at weaning.
[h]Analysis of body weight at attainment and body weight at weaning for both linear trend and pairwise comparisons were performed using mixed effects models with exposure concentration as a covariate, litter as a random effect, and a Dunnett-Hsu adjustment for multiple pairwise comparisons.

Several significant organ weight changes were observed but were considered related to reduced growth and not toxicologically relevant (Appendix E).

At study termination, erythrocyte count was significantly increased in the ≥125 mg/L male rats and in the 250 mg/L female rats (Table 13). Compared to the control groups, erythrocyte count was approximately 5% higher in the 250 mg/L male and female rats and 7% higher in the 500 mg/L male rats, indicating erythrocytosis. In male rats, the mean cell volume (MCV) was significantly decreased in the ≥125 mg/L groups, whereas in female rats, the MCV was significantly decreased in the 500 mg/L group. Compared to the control groups, MCV was approximately 7% and 4% lower in the 500 mg/L male and female rats, respectively. The decreases in MCV indicated an erythrocyte microcytosis. Additionally, in male rats, the mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) were significantly decreased in the ≥125 mg/L and 500 mg/L groups, respectively. In female rats, the MCH and MCHC were significantly decreased in the ≥250 mg/L and 500 mg/L groups, respectively. At

PND 28, there were a number of low magnitude, statistically significant changes in male and/or female rats that were consistent with changes at study termination, demonstrating the beginning of an exposure-related progressive microcytic erythrocytosis (Appendix E).

Table 13. Summary of Select Hematology Data for Male and Female Rats in the Perinatal and Three-month Drinking Water Study of Sodium Metavanadate

Endpoint[a,b]	0 mg/L	31.3 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L
Male						
n	10	9	10	9	10	12[c]
Hematocrit (%)	47.3 ± 0.6	47.8 ± 0.7	46.7 ± 0.7	47.2 ± 0.8	47.7 ± 0.5	47.0 ± 0.8
Hemoglobin (g/dL)	15.0 ± 0.1*	15.2 ± 0.2	15.0 ± 0.2	14.8 ± 0.2	15.0 ± 0.1	14.5 ± 0.2
Erythrocytes (10^6/µL)	8.61 ± 0.09**	8.75 ± 0.12	8.75 ± 0.08	8.96 ± 0.09*	9.04 ± 0.09**	9.23 ± 0.18**
Mean Cell Volume (fL)	54.9 ± 0.5**	54.7 ± 0.5	53.4 ± 0.5	52.7 ± 0.7*	52.8 ± 0.3**	51.0 ± 0.4**
Mean Cell Hemoglobin (pg)	17.4 ± 0.1**	17.4 ± 0.1	17.1 ± 0.1	16.6 ± 0.2**	16.6 ± 0.1**	15.8 ± 0.1**
Mean Cell Hemoglobin Concentration (g/dL)	31.7 ± 0.2**	31.7 ± 0.2	32.0 ± 0.2	31.4 ± 0.1	31.4 ± 0.1	30.9 ± 0.1**
Female						
n	10	10	9	9	8	10[c]
Hematocrit (%)	44.1 ± 0.8	45.0 ± 0.4	42.8 ± 0.8	43.4 ± 0.5	45.6 ± 0.5	43.3 ± 0.6
Hemoglobin (g/dL)	14.2 ± 0.2	14.5 ± 0.1	13.7 ± 0.4	14.1 ± 0.1	14.4 ± 0.1	13.6 ± 0.2
Erythrocytes (10^6/µL)	7.78 ± 0.10	8.02 ± 0.06	7.77 ± 0.16	7.96 ± 0.13	8.18 ± 0.10*	7.97 ± 0.10
Mean Cell Volume (fL)	56.7 ± 0.5**	56.2 ± 0.5	55.2 ± 0.5	54.7 ± 0.7	55.8 ± 0.5	54.3 ± 0.4**
Mean Cell Hemoglobin (pg)	18.2 ± 0.1**	18.1 ± 0.2	17.7 ± 0.3	17.7 ± 0.3	17.6 ± 0.1*	17.0 ± 0.1**
Mean Cell Hemoglobin Concentration (g/dL)	32.2 ± 0.2**	32.3 ± 0.2	32.0 ± 0.4	32.4 ± 0.2	31.6 ± 0.2	31.4 ± 0.2*

Statistical significance for an exposed 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 are presented as mean ± standard error.
[b]Statistical analysis performed by the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.
[c]Group contained multiple animals per litter.

Urea nitrogen was significantly increased in both the ≥125 mg/L male groups and in the ≥250 mg/L female groups (Table 14). These changes were mild and consistent with dehydration and supported by higher urine specific gravity in the respective exposed groups (Appendix E). In both male and female rats, globulin concentrations were significantly decreased resulting in a significant increase in the albumin to globulin (A/G) ratio; these changes were observed in the ≥250 mg/L male groups and in the 500 mg/L female group. Compared to the control groups, globulin concentrations were approximately 19% and 14% lower in the 500 mg/L male and female rats, respectively. Cholesterol concentrations were significantly decreased in the ≥125 mg/L male groups and the ≥250 mg/L female groups. The alkaline phosphatase activity was mildly but significantly decreased in both the ≥250 mg/L male groups and in the ≥125 mg/L female groups. Alanine aminotransferase activity was mildly but significantly increased in the ≥125 mg/L female groups.

Table 14. Summary of Select Clinical Chemistry Data for Male and Female Rats in the Perinatal and Three-month Drinking Water Study of Sodium Metavanadate

Endpoint[a,b]	0 mg/L	31.3 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L[c]
Male						
n	10	10	10	10	10	12
Urea Nitrogen (mg/dL)	16.0 ± 0.4**[d]	15.4 ± 0.5	17.1 ± 0.7[d]	18.1 ± 0.8*[e]	23.0 ± 0.8**[e]	30.9 ± 1.0**[f]
Albumin (g/dL)	4.61 ± 0.08*	4.55 ± 0.03	4.44 ± 0.04	4.57 ± 0.10	4.71 ± 0.05	4.77 ± 0.07
Globulin (g/dL)	2.50 ± 0.08**[d]	2.27 ± 0.04	2.37 ± 0.08[d]	2.39 ± 0.09[e]	2.19 ± 0.07**[d]	2.03 ± 0.03**[f]
A/G Ratio	1.84 ± 0.06**[d]	2.01 ± 0.04	1.89 ± 0.08[d]	1.94 ± 0.04[e]	2.16 ± 0.07**[d]	2.36 ± 0.03**[f]
Cholesterol (mg/dL)	141.6 ± 5.6**[e]	134.4 ± 3.7	132.8 ± 5.0[d]	125.2 ± 4.7*	125.1 ± 7.0*	111.6 ± 2.8**[g]
Alkaline Phosphatase (IU/L)	128.9 ± 5.1**[d]	121.7 ± 5.1	128.8 ± 7.8[d]	123.7 ± 6.7	99.8 ± 5.8**	96.9 ± 4.0**[h]
Female						
n	10	10	10	10	10	10
Urea Nitrogen (mg/dL)	15.7 ± 0.7**	15.5 ± 0.3	17.8 ± 0.9	16.6 ± 0.7	20.1 ± 0.7**	26.2 ± 0.7**
Albumin (g/dL)	4.86 ± 0.05	4.89 ± 0.07	4.92 ± 0.06	4.69 ± 0.07	4.94 ± 0.06	4.77 ± 0.06
Globulin (g/dL)	1.90 ± 0.04**	1.86 ± 0.06	1.82 ± 0.06	1.87 ± 0.06	1.78 ± 0.05	1.63 ± 0.05**
A/G Ratio	2.57 ± 0.04**	2.66 ± 0.11	2.74 ± 0.13	2.53 ± 0.09	2.80 ± 0.10	2.94 ± 0.08**
Cholesterol (mg/dL)	119.1 ± 4.0**	116.5 ± 3.2	127.3 ± 4.9	114.1 ± 5.1	108.3 ± 2.3*	96.1 ± 2.9**
Alkaline Phosphatase (IU/L)	112.2 ± 3.2**	103.1 ± 6.3	106.8 ± 9.4	87.5 ± 5.1**	74.8 ± 4.8**	82.0 ± 4.1**
Alanine Aminotransferase (IU/L)	41.0 ± 2.4**	47.4 ± 2.7	45.1 ± 2.3	51.5 ± 3.2*	50.4 ± 1.7**	57.4 ± 3.2**

Statistical significance for an exposed 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 are presented as mean ± standard error.
[b]Statistical analysis performed by the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.
[c]Group contained multiple animals per litter. Litter-based methods were not used in the analysis.
[d]n = 9. One sample in the indicated group was excluded from analysis due to biological implausibility.
[e]n = 8. Two samples in the indicated group were excluded from analysis due to biological implausibility.
[f]n = 9. Three samples in the indicated group were excluded from analysis due to biological implausibility.
[g]n = 10. Two samples in the indicated group were excluded from analysis due to biological implausibility.
[h]n = 11. One sample in the indicated group was excluded from analysis due to biological implausibility.

Rats exposed to 500 mg/L sodium metavanadate displayed a small (10%) yet significant decrease in cauda epididymal weight (Appendix E). However, there were no correlating histopathological findings in either the testes or epididymides. Analysis of female vaginal cytology (0, 125, and 250 mg/L groups) indicated that sodium metavanadate did not affect the number of estrous cycles or overall estrous cycle length (Appendix E). Although the Markov model estimates of stage length indicated that the length of proestrus was slightly but significantly decreased in the 500 mg/L group, this was likely a spurious response given that the length of time in proestrus was lowest in 125 mg/L group and the length of time in the control group was longer than usually observed in higher powered NTP studies.[71; 72]

Histopathology

This section describes the statistically significant or biologically noteworthy changes in the incidence of gross lesions and nonneoplastic lesions in the small and large intestines.

Small and large intestines: Histopathologically, significantly increased incidences of epithelial hyperplasia were observed in the epithelium of the small (ileum, jejunum, and duodenum) intestine, and higher incidences were observed in the large (cecum, colon, and rectum) intestine (Table 15). This lesion was most prevalent in the ileum, and the next highest incidences were seen in the jejunum. In both of these segments of the small intestine in male rats, this lesion was seen in animals exposed to ≥125 mg/L, with a significant trend and pairwise comparisons in the ileum of males exposed to ≥125 mg/L and in the jejunum of males exposed to ≥250 mg/L. Epithelial hyperplasia was seen in the duodenum of only one male in the 500 mg/L group. The severity of epithelial hyperplasia in the small intestine was generally minimal to mild and increased slightly with exposure concentration. Severities were similar in female rats; however, epithelial hyperplasia was not seen in the jejunum in the 250 mg/L female group and did not occur in the duodenum of female rats. Epithelial hyperplasia was observed in the ileum of females exposed to ≥125 mg/L, with a positive trend and significant increases in the ≥250 mg/L groups. In the jejunum, epithelial hyperplasia was observed in only one female exposed to 125 mg/L; a positive trend and a significant increase were observed in females exposed to 500 mg/L.

Epithelial hyperplasia in the small intestine was characterized by elongated crypts and shortened villi. Crypts were lined by crowded epithelial cells with basophilic cytoplasm and elongated nuclei. There was also a paucity of Paneth cells and eosinophilic granules at the base. Villi had increased numbers of basophilic epithelial cells that formed multiple layers of epithelial cells and irregular fronds and blebs extending into the lumen (Figure 2).

The only exposure-related gross lesion observed was mucoid contents in the cecum of one male rat that was euthanized moribund (Appendix E).

Epithelial hyperplasia of the cecum was seen in the 250 and 500 mg/L male groups and in the rectum of one male in the 500 mg/L group (Table 15). The severity of this lesion was generally minimal to mild and increased slightly with exposure concentration. A male rat that was euthanized moribund also had minimal necrosis in the cecum, which correlated with the mucoid contents gross lesion. Severities of epithelial hyperplasia in the large intestine of females were similar to those of males; however, epithelial hyperplasia was seen only in the 500 mg/L group, in the cecum, rectum, and colon.

Microscopic findings in the large intestine consisted of elongated crypts lined by basophilic epithelial cells with an irregular luminal surface characterized by increased numbers of basophilic epithelial cells that occasionally formed multiple layers of epithelial cells and/or irregular fronds and blebs extending into the lumen. In the colon, the surface had a wavy appearance due to the elongation of some of the hyperplastic crypts.

Incidences of epithelial hyperplasia at all intestinal sites (combined) were observed in males and females exposed to ≥125 mg/L, with a significant trend and pairwise comparisons. The lesion occurred in the ileum of all 500 mg/L animals, except for one male. Epithelial hyperplasia was not observed in males or females exposed to ≤62.5 mg/L.

Table 15. Incidences of Epithelial Hyperplasia of the Large and Small Intestines in Male and Female Rats in the Perinatal and Three-month Drinking Water Study of Sodium Metavanadate

	0 mg/L	31.3 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L[a]
Male						
n[b]	10	10	10[c]	10[c]	10[d]	13
Intestine, Small, Ileum						
Epithelium, hyperplasia[e]	0**	0	0	5* (1.4)[f]	9** (1.3)	12** (2.1)
Intestine, Small, Jejunum						
Epithelium, hyperplasia	0**	–[g]	0	1 (1.0)	6** (1.0)	7** (1.4)
Intestine, Small, Duodenum						
Epithelium, hyperplasia	0	–	–	–	0	1 (1.0)
Intestine, Large, Cecum						
Epithelium, hyperplasia	0	0	0	0	2 (1.0)	1 (2.0)
Intestine, Large, Rectum						
Epithelium, hyperplasia	0	0	0	0	0	1 (1.0)
Intestine, Any Site						
Epithelium, hyperplasia	0**	0	0	5*	9**	13**
Female						
n	10[h]	0	10	10	10	12
Intestine, Small, Ileum						
Epithelium, hyperplasia	0**	–	0	4 (1.3)	9** (1.6)	12** (2.1)
Intestine, Small, Jejunum						
Epithelium, hyperplasia	0**	–	–	1 (1.0)	0	6* (1.3)
Intestine, Large, Cecum						
Epithelium, hyperplasia	0	–	–	0	0	2 (2.0)
Intestine, Large, Rectum						
Epithelium, hyperplasia	0	–	–	0	0	1 (2.0)
Intestine, Large, Colon						
Epithelium, hyperplasia	0	–	–	0	0	2 (2.0)
Intestine, Any Site						
Epithelium, hyperplasia	0**	–	0	4*	9**	12**

Statistical significance for an exposed 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]Group contained multiple animals per litter. Litter-based methods were not used in the analysis.
[b]Number of animals examined microscopically.
[c]For the small intestine, ileum data, n = 9 for the male 62.5 and 125 mg/L groups.
[d]For the large intestine, rectum data, n = 9 for the male 250 mg/L group.
[e]Number of animals with lesion. Statistical analyses performed by Cochran-Armitage (trend) and Fisher's exact (pairwise) one-sided tests.
[f]Average severity grade of observed lesion in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.
[g]Data were not collected for an exposed group when animals exposed to higher concentrations exhibited no incidences of nonneoplastic lesions in that tissue.
[h]For the small intestine, ileum data, n = 9 for the 0 mg/L female group.

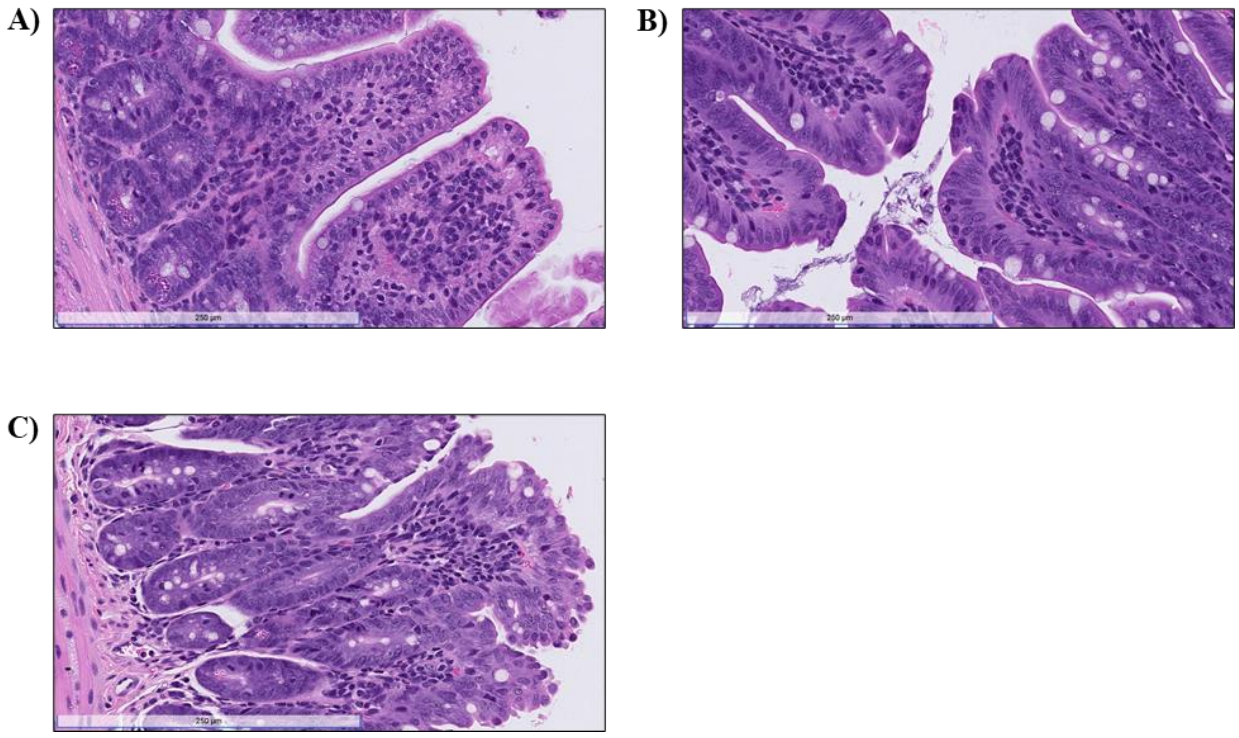


Figure 2. Representative Images of Control Epithelium and Epithelial Hyperplasia of the Intestines of Rodents in the Three-month Drinking Water Studies of Sodium Metavanadate and Vanadyl Sulfate (H&E)

(A) Morphology of the epithelium of the ileum in a male control mouse. (B) Moderate epithelial hyperplasia in the ileum of a male mouse exposed to 335 mg/L vanadyl sulfate. (C) Marked epithelial hyperplasia in the ileum of a female mouse exposed to 335 mg/L vanadyl sulfate. H&E = hematoxylin and eosin stain.

Mice

Three-month Study

There was no effect on survival of male or female mice due to sodium metavanadate exposure. One 500 mg/L male mouse was removed from the study during week 3 due to clinical observations of ruffled coat, lethargy, hunched, and cold to touch, although it is unclear whether these can be fully attributed to sodium metavanadate exposure (Appendix E). One female 250 mg/L mouse was removed in week 5 due to a broken hind limb. Body weights of male mice in the 250 and 500 mg/L groups were significantly decreased relative to the control group for most of the study (Table 16; Figure 3). Although significant, the body weight of the 250 mg/L group was within 11% of that of the control group throughout the study. Terminal body weight of the 500 mg/L male group was 26% lower than that of the control group; mice in this group lost an average of 6% body weight in the first 3 weeks of the study and then continually gained weight for the remaining duration. Similarly, female mice in the 500 mg/L group had significantly decreased body weights at every interval after study day 0, with a terminal body weight 23% lower than that of the control group (Table 17; Figure 3). The 250 mg/L female body weight was 14% lower than that of the control group at study termination but was generally similar to the control group throughout. No body weight changes were seen in males or females exposed to ≤125 mg/L sodium metavanadate.

Table 16. Summary of Survival and Body Weights of Male Mice in the Three-month Drinking Water Study of Sodium Metavanadate

Study Day[a]	0 mg/L		31.3 mg/L			62.5 mg/L			125 mg/L			250 mg/L			500 mg/L		
	Wt. (g)[b,c]	n	Wt. (g)	Wt. (% of Controls)	n	Wt. (g)	Wt. (% of Controls)	n	Wt. (g)	Wt. (% of Controls)	n	Wt. (g)	Wt. (% of Controls)	n	Wt. (g)	Wt. (% of Controls)	n
0	23.0 ± 0.4	10	23.3 ± 0.3	101.4	10	23.2 ± 0.3	101.2	10	23.2 ± 0.4	101.2	10	23.4 ± 0.2	102.0	10	23.4 ± 0.2	101.9	10
7	25.0 ± 0.3**	10	25.2 ± 0.3	100.8	10	24.9 ± 0.3	99.6	10	25.1 ± 0.3	100.4	10	24.3 ± 0.3	97.0	10	21.9 ± 0.4**	87.5	10
14	26.2 ± 0.3**	10	26.5 ± 0.3	101.1	10	26.0 ± 0.4	99.2	10	26.3 ± 0.4	100.2	10	25.4 ± 0.3	96.9	10	21.4 ± 0.7**	81.6	9[d]
21	27.8 ± 0.3**	10	27.9 ± 0.4	100.1	10	27.3 ± 0.4	98.1	10	27.6 ± 0.5	99.4	10	26.2 ± 0.3**	94.2	10	22.0 ± 0.6**	79.1	9
28	29.0 ± 0.4**	10	29.4 ± 0.6	101.3	9[d]	28.5 ± 0.4	98.1	10	28.9 ± 0.5	99.4	10	27.0 ± 0.4**	93.1	10	23.7 ± 0.5**	81.6	9
35	30.5 ± 0.5**	10	30.7 ± 0.7	100.8	10	29.9 ± 0.4	97.9	10	30.5 ± 0.6	100.0	10	28.4 ± 0.5**	93.0	10	24.9 ± 0.6**	81.7	9
42	32.2 ± 0.9**	10	32.7 ± 0.7	101.6	10	31.7 ± 0.4	98.4	10	32.0 ± 0.7	99.6	10	29.6 ± 0.5*	92.1	10	26.4 ± 0.5**	82.3	9
49	34.3 ± 0.7**	10	34.5 ± 0.9	100.7	10	33.2 ± 0.5	96.8	10	33.8 ± 0.9	98.7	10	30.6 ± 0.5**	89.3	10	27.2 ± 0.5**	79.5	9
56	35.0 ± 0.8**	10	35.7 ± 0.9	102	10	34.3 ± 0.7	98.1	10	35.2 ± 1.0	100.6	10	31.5 ± 0.7**	90.2	10	27.8 ± 0.6**	79.4	9
63	36.7 ± 1.0**	10	37.5 ± 0.9	102.4	10	36.5 ± 0.7	99.6	10	37.3 ± 1.1	101.8	10	33.1 ± 0.6**	90.2	10	28.9 ± 0.5**	78.8	9
70	37.6 ± 1.0**	10	38.9 ± 1.1	103.2	10	37.2 ± 0.8	98.8	10	38.0 ± 1.0	100.9	10	34.1 ± 0.7**	90.5	10	29.0 ± 0.5**	77.1	9
77	38.5 ± 1.1**	10	39.9 ± 1.2	103.6	10	38.7 ± 0.9	100.4	10	39.3 ± 1.2	102.0	10	34.7 ± 0.7**	90.1	10	29.3 ± 0.6**	75.9	9
84	39.9 ± 1.2**	10	41.5 ± 1.3	104.0	10	40.1 ± 1.0	100.6	10	40.6 ± 1.2	101.7	10	35.8 ± 0.8**	89.8	10	30.0 ± 0.6**	75.2	9
91	41.9 ± 1.1**	10	43.3 ± 1.2	103.2	10	42.4 ± 1.1	101	10	42.7 ± 1.1	101.7	10	37.5 ± 0.9**	89.5	10	31.2 ± 0.7**	74.3	9

Statistical significance for an exposed 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]Study day 0 is the day animals were placed on study.
[b]Statistical analysis performed by the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.
[c]Weights shown are mean ± standard error.
[d]One animal weight was excluded as an outlier and thus excluded from analysis on the indicated study day.

Table 17. Summary of Survival and Body Weights of Female Mice in the Three-month Drinking Water Study of Sodium Metavanadate

Study Day[a]	0 mg/L		31.3 mg/L			62.5 mg/L			125 mg/L			250 mg/L			500 mg/L		
	Wt. (g)[b,c]	n	Wt. (g)	Wt. (% of Controls)	n	Wt. (g)	Wt. (% of Controls)	n	Wt. (g)	Wt. (% of Controls)	n	Wt. (g)	Wt. (% of Controls)	n	Wt. (g)	Wt. (% of Controls)	n
0	18.4 ± 0.3	10	18.4 ± 0.4	99.6	10	18.6 ± 0.3	101.1	10	18.6 ± 0.2	100.8	10	18.8 ± 0.3	102.0	10	18.7 ± 0.3	101.2	10
7	19.1 ± 0.4**	10	19.0 ± 0.3	99.1	10	19.2 ± 0.2	100.3	10	18.5 ± 0.2	96.9	10	19.0 ± 0.2	99.1	10	17.7 ± 0.3**	92.3	10
14	20.4 ± 0.4**	10	20.9 ± 0.4	102.2	10	20.7 ± 0.4	101.4	10	20.2 ± 0.3	98.8	10	20.0 ± 0.2	97.8	10	19.0 ± 0.2**	93.1	10
21	21.5 ± 0.3**	10	21.6 ± 0.3	100.4	10	21.7 ± 0.4	100.9	10	21.5 ± 0.2	100	10	20.9 ± 0.3	97.1	10	20.3 ± 0.3*	94.5	10
28	22.3 ± 0.5**	9[d]	22.1 ± 0.5	99.2	10	22.3 ± 0.5	100.1	10	22.2 ± 0.4	99.7	10	21.6 ± 0.3	97.1	10	20.9 ± 0.2*	93.9	10
35	23.5 ± 0.4**	10	23.6 ± 0.5	100.2	10	24.3 ± 0.7	103.1	10	22.7 ± 0.4	96.3	10	22.6 ± 0.2	96.0	9	21.6 ± 0.3**	91.6	10
42	24.2 ± 0.4**	10	24.5 ± 0.6	101.5	10	25.3 ± 0.7	104.8	10	24.0 ± 0.4	99.3	10	23.2 ± 0.5	95.9	9	22.7 ± 0.3*	93.8	10
49	25.2 ± 0.7**	10	25.6 ± 0.6	101.4	10	27.1 ± 0.8	107.4	10	25.4 ± 0.6	100.5	10	24.6 ± 0.7	97.5	9	23.3 ± 0.2*	92.2	10
56	25.8 ± 0.7**	10	26.7 ± 0.8	103.5	10	28.2 ± 1.1	109.2	10	25.9 ± 0.8	100.4	10	24.7 ± 0.6	95.6	9	23.1 ± 0.2*	89.4	10
63	27.3 ± 1.0**	10	27.7 ± 0.8	101.5	10	29.6 ± 1.2	108.4	10	26.7 ± 1.0	97.7	10	25.4 ± 0.7	93.0	9	23.5 ± 0.3**	86.2	10
70	29.2 ± 1.1**	10	29.8 ± 0.9	102.2	10	31.1 ± 1.5	106.6	10	28.6 ± 0.8	97.9	10	25.9 ± 0.6*	88.8	9	24.3 ± 0.2**	83.3	10
77	29.3 ± 1.0**	10	30.8 ± 0.8	105.1	10	32.1 ± 1.3	109.6	10	28.5 ± 0.9	97.4	10	26.7 ± 0.7	91.1	9	24.1 ± 0.2**	82.2	10
84	30.8 ± 1.2**	10	32.8 ± 1.0	106.5	10	33.0 ± 1.7	107.2	10	29.8 ± 1.0	96.9	10	26.7 ± 0.8*	86.8	8[d]	24.5 ± 0.3**	79.5	10
91	32.2 ± 1.2**	10	33.9 ± 1.0	105.2	10	34.0 ± 1.6	105.6	10	30.9 ± 1.0	96.0	10	27.6 ± 0.9**	85.7	9	24.9 ± 0.3**	77.2	10

Statistical significance for an exposed 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]Study day 0 is the day animals were placed on study.
[b]Statistical analysis performed by the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.
[c]Weights shown are mean ± standard error.
[d]One animal weight was excluded as an outlier and thus excluded from analysis on the indicated study day.

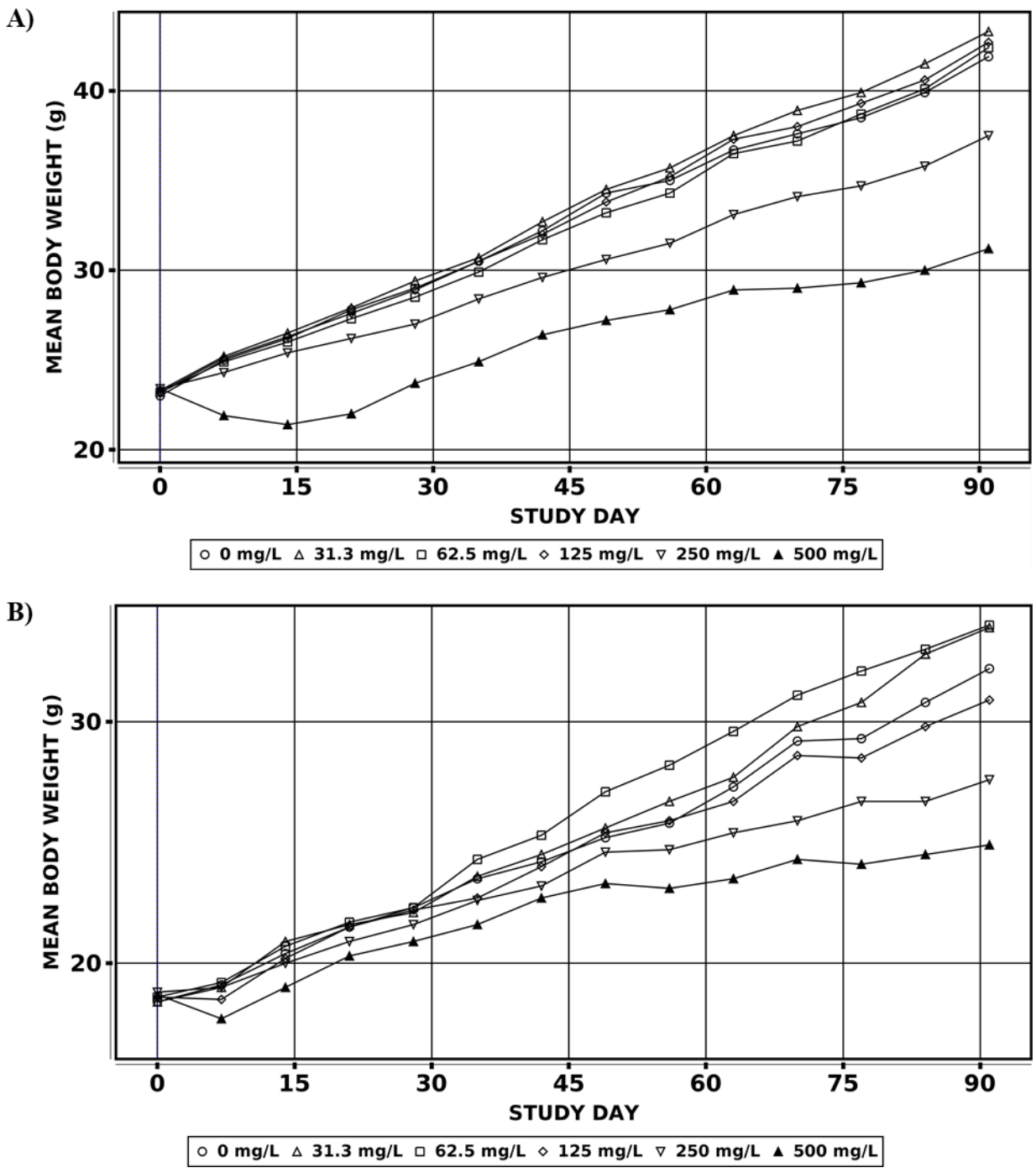


Figure 3. Growth Curves for Male and Female Mice in the Three-month Drinking Water Study of Sodium Metavanadate

Growth curves are shown for (A) males and (B) females.

In male and female mice, average water consumption during the 3-month study (study day 0–91) was 15% lower in the 500 mg/L groups than in the control groups; in all other exposed groups, it was within 5% of the control groups (Table 18). Sodium metavanadate intake over the course of the study (study day 0–91) is presented in Table 18.

Table 18. Summary of Water and Sodium Metavanadate Consumption by Male and Female Mice in the Three-month Drinking Water Study

Study Day[a]	0 mg/L	31.3 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L
Male (g/kg/day)[b]						
0–7	160.9 ± 7.2** (10)	148.8 ± 3.2 (10)	156.6 ± 4.5 (10)	146.7 ± 4.5 (10)	135.1 ± 4.4** (10)	96.7 ± 3.3** (10)
21–28	150.6 ± 18.2 (10)	121.5 ± 1.7 (8)	136.2 ± 7.4 (10)	122.2 ± 4.8 (8)	134.4 ± 9.2 (10)	116.1 ± 11.0 (8)
84–91	86.2 ± 4.0 (10)	81.6 ± 3.0 (10)	80.8 ± 3.6 (10)	79.2 ± 3.8 (10)	87.8 ± 3.0 (10)	81.2 ± 2.6 (9)
0–91	110.2 ± 4.0** (10)	105.6 ± 2.8 (10)	107.7 ± 3.4 (10)	104.7 ± 3.9 (10)	106.6 ± 2.9 (10)	93.2 ± 2.2** (9)
Chemical Intake (mg/kg/day)[c,d]						
0–91	0.0 ± 0.0 (10)	3.3 ± 0.1 (10)	6.7 ± 0.2 (10)	13.1 ± 0.5 (10)	26.6 ± 0.7 (10)	46.6 ± 1.1 (9)
Female (g/kg/day)						
0–7	136.4 ± 2.9** (10)	116.7 ± 3.5 (10)	142.1 ± 2.2 (10)	125.9 ± 1.2* (10)	97.9 ± 1.1** (10)	67.7 ± 1.2** (10)
21–28	128.5 ± 4.0** (10)	107.8 ± 3.0 (10)	131.1 ± 2.7 (10)	122.1 ± 1.9 (10)	111.3 ± 2.0** (10)	91.6 ± 0.9** (10)
84–91	97.2 ± 5.6 (10)	84.0 ± 2.5 (10)	78.2 ± 3.7* (10)	92.4 ± 2.7 (10)	101.2 ± 3.3 (9)	85.4 ± 2.2 (10)
0–91	107.8 ± 2.8** (10)	103.2 ± 2.0 (10)	107.9 ± 3.1 (10)	108.3 ± 1.9 (10)	103.8 ± 1.9 (9)	91.2 ± 0.8** (10)
Chemical Intake (mg/kg/day)						
0–91	0.0 ± 0.0 (10)	3.2 ± 0.1 (10)	6.7 ± 0.2 (10)	13.5 ± 0.2 (10)	25.9 ± 0.5 (9)	45.6 ± 0.4 (10)

Statistical significance for an exposed 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 are presented as mean ± standard error (number of animals).
[b]Each exposed 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.
[c]Chemical intake calculated as: ([exposure concentration × water consumption]/[average body weight of day range]).
[d]No statistical analysis was performed on the chemical intake data.

Absolute thymus weights were lower in the ≥62.5 mg/L male and ≥125 mg/L female groups compared to those of the control groups, although only the decreases in the ≥250 mg/L male groups and 500 mg/L female group were significant (Table 19). Relative thymus weights were lower in the ≥62.5 mg/L male groups, although none of these decreases were significant. Relative thymus weights were lower in the 62.5, 125, and 500 mg/L female groups, although none of these decreases were significant. These decreases were attributed to exposure to sodium metavanadate. Several other significant organ weight changes were considered secondary to body weight effects and not toxicologically relevant (Appendix E).

Table 19. Summary of Thymus Weights and Thymus-Weight-to-Body-Weight Ratios of Male and Female Mice in the Three-month Drinking Water Study of Sodium Metavanadate

Endpoint[a,b]	0 mg/L	31.3 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L
Male						
n	10	10	10[c]	10	10	9
Terminal Body Wt. (g)	41.9 ± 1.1**	43.3 ± 1.2	42.4 ± 1.1	42.7 ± 1.1	37.5 ± 0.9**	31.2 ± 0.7**
Thymus						
Absolute (g)	0.065 ± 0.007**	0.071 ± 0.007	0.059 ± 0.005	0.059 ± 0.007	0.047 ± 0.003*	0.042 ± 0.002**
Relative (mg/g)[d]	1.53 ± 0.12*	1.65 ± 0.15	1.37 ± 0.09	1.37 ± 0.13	1.24 ± 0.07	1.34 ± 0.07
Female						
n	10	10	10	10	9	10
Terminal Body Wt. (g)	32.2 ± 1.2**	33.9 ± 1.0	34.0 ± 1.6	30.9 ± 1.0	27.6 ± 0.9**	24.9 ± 0.3**
Thymus						
Absolute (g)	0.060 ± 0.003**	0.069 ± 0.005	0.061 ± 0.003	0.053 ± 0.002	0.052 ± 0.003	0.045 ± 0.002**
Relative (mg/g)	1.85 ± 0.07	2.02 ± 0.12	1.81 ± 0.07	1.71 ± 0.04	1.88 ± 0.07	1.79 ± 0.09

Statistical significance for an exposed 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 are presented as mean ± standard error.
[b]Statistical analysis performed by the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.
[c]One animal's thymus weight was excluded as an outlier and thus excluded from analysis.
[d]Relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight.

In both male and female mice, the erythrocyte count was significantly increased in the ≥125 mg/L groups, and the reticulocyte counts were significantly increased in the ≥250 mg/L male and female groups (Table 20). Compared to the control groups, erythrocyte counts were approximately 17% and 25% higher in the 500 mg/L male and female mice, respectively. In male mice, MCV was significantly decreased in all exposed groups, whereas in female mice MCV was significantly decreased in the ≥62.5 mg/L groups. Compared to the control groups, MCV was approximately 18% and 21% lower in the 500 mg/L male and female mice, respectively. The decreases in MCV indicated erythrocyte microcytosis. In contrast to the increased erythrocyte count, other markers of the erythron (hematocrit and hemoglobin) demonstrated decreases. For example, hemoglobin concentration was significantly decreased in the ≥125 mg/L male groups and 500 mg/L females. In males, despite the significant increase in erythrocyte count, these smaller erythrocytes (microcytosis) led to significant decreases in the hematocrit in the 250 mg/L and 500 mg/L groups. Additionally, MCH was significantly decreased in the ≥62.5 mg/L male and female mice.

Table 20. Summary of Select Hematology Data for Male and Female Mice in the Three-month Drinking Water Study of Sodium Metavanadate

Endpoint[a,b]	0 mg/L	31.3 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L
Male						
n	10	10	9	10	10	9
Hematocrit (%)	53.6 ± 0.4**	54.1 ± 0.3	53.5 ± 0.4	52.4 ± 0.3	52.0 ± 0.6*	51.4 ± 0.5**
Hemoglobin (g/dL)	15.2 ± 0.1**	15.5 ± 0.1	15.1 ± 0.1	14.7 ± 0.1**	14.2 ± 0.2**	13.7 ± 0.1**
Erythrocytes (10^6/µL)	10.50 ± 0.08**	10.74 ± 0.05	10.71 ± 0.08	10.86 ± 0.11**	11.60 ± 0.11**	12.28 ± 0.11**
Reticulocytes (10^3/µL)	267.0 ± 7.0**	262.7 ± 5.6	262.6 ± 6.7	273.1 ± 5.2	299.8 ± 9.7*	368.2 ± 9.2**
Mean Cell Volume (fL)	51.1 ± 0.2**	50.4 ± 0.2*	50.0 ± 0.2**	48.3 ± 0.2**	44.9 ± 0.2**	41.9 ± 0.4**
Mean Cell Hemoglobin (pg)	14.5 ± 0.1**	14.4 ± 0.1	14.1 ± 0.1**	13.5 ± 0.1**	12.3 ± 0.1**	11.1 ± 0.1**
Female						
n	10	9	9	9	9	10
Hematocrit (%)	54.2 ± 0.7	55.0 ± 1.0	53.3 ± 0.7	53.9 ± 0.6	54.7 ± 1.2	53.6 ± 0.7
Hemoglobin (g/dL)	15.7 ± 0.2**	15.3 ± 0.6	15.4 ± 0.2	15.2 ± 0.1	14.9 ± 0.3	14.3 ± 0.2**
Erythrocytes (10^6/µL)	10.58 ± 0.17**	10.81 ± 0.18	10.77 ± 0.14	11.57 ± 0.12**	12.57 ± 0.19**	13.25 ± 0.19**
Reticulocytes (10^3/µL)	310.8 ± 15.0**	280.9 ± 19.1	268.5 ± 17.2	352.9 ± 16.1	406.8 ± 14.2**	465.7 ± 13.6**
Mean Cell Volume (fL)	51.3 ± 0.3**	50.9 ± 0.3	49.5 ± 0.2**	46.6 ± 0.1**	43.5 ± 0.4**	40.5 ± 0.3**
Mean Cell Hemoglobin (pg)	14.8 ± 0.1**	14.1 ± 0.4	14.3 ± 0.1**	13.2 ± 0.1**	11.9 ± 0.1**	10.8 ± 0.2**

Statistical significance for an exposed 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 are presented as mean ± standard error.
[b]Statistical analysis performed by the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.

Male mice in the 500 mg/L group displayed significantly higher testicular spermatid counts and counts per gram of testis (39%–41%) (Table 21). Cauda epididymal weights were also significantly lower (12%), and sperm counts appeared lower (12%) but were not statistically significant. There were no correlating histopathological findings in the testis or epididymis that were clearly attributable to sodium metavanadate exposure. Female mice exposed to sodium metavanadate did not display any alterations in estrous cyclicity that were attributed to exposure (Appendix E).

Table 21. Summary of Reproductive Tissue Evaluations for Male Mice in the Three-month Drinking Water Study of Sodium Metavanadate

Endpoint[a]	0 mg/L	125 mg/L	250 mg/L	500 mg/L
n	10	10	10	9
Weight (g)[b]				
Cauda epididymis	0.017 ± 0.001**	0.018 ± 0.001	0.016 ± 0.001	0.015 ± 0.000*
Spermatid Measurements[c]				
Spermatid heads (10^6/g testis)	103.8 ± 11.3**	99.2 ± 7.1	123.0 ± 10.6	146.6 ± 8.7**
Spermatid heads (10^6/testis)	9.3 ± 0.9**	9.5 ± 0.8	10.7 ± 1.2	12.9 ± 0.9*
Epididymal Spermatozoal Measurements				

Endpoint[a]	0 mg/L	125 mg/L	250 mg/L	500 mg/L
Sperm motility (%)	85.2 ± 1.9	86.2 ± 1.8	84.1 ± 1.6	87.8 ± 1.6
Sperm (10^3/mg cauda epididymis)	1,981.7 ± 149.4	1,766.4 ± 80.7	2,034.2 ± 229.8	2,036.9 ± 85.3
Cauda epididymis sperm count (millions)	34.5 ± 2.9	30.9 ± 1.5	31.8 ± 3.5	30.5 ± 1.3

Statistical significance for an exposed 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 are presented as mean ± standard error.
[b]Statistical analysis performed by the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.
[c]Statistical analysis performed by the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.

Histopathology

This section describes the statistically significant or biologically noteworthy changes in the incidence of nonneoplastic lesions in the small intestine, large intestine, and kidney.

There were no gross lesions attributable to sodium metavanadate exposure in mice.

Small and large intestines: Epithelial hyperplasia was observed in the small and large intestines in male mice and in the small intestine in female mice (Table 22). These lesions were not observed in the control groups. Hyperplasia of the small intestinal epithelium in male mice was seen only in the ileum. It was observed in male mice exposed to ≥125 mg/L, with a positive trend and significant pairwise comparisons in the ≥250 mg/L groups, and the severity ranged from minimal to mild. In the large intestine, this lesion was seen in the colon of one high-exposure animal. This animal was euthanized moribund on study day 20 and exhibited erosion of the cecal epithelium. Epithelial hyperplasia was also seen in the colon of one male in the 500 mg/L group. Hyperplasia of the small intestinal epithelium in female mice was seen in the ileum and jejunum. In the ileum, epithelial hyperplasia was observed in female mice exposed to ≥125 mg/L, with a positive trend and significant pairwise comparisons in the ≥250 mg/L groups, and the severity ranged from minimal to mild. In the jejunum, epithelial hyperplasia was observed in one female in the 500 mg/L group. Incidences of epithelial hyperplasia in all intestinal sites (combined) were observed in males and females in the ≥125 mg/L groups, with a positive trend and significant pairwise comparisons in the ≥250 mg/L groups. This lesion was not observed in males and females exposed to ≤62.5 mg/L. Hyperplasia of the intestinal epithelium in mice was nearly identical to that described in rats exposed to sodium metavanadate (see Sodium Metavanadate Rat Histopathology).

Table 22. Incidences of Epithelial Hyperplasia of the Small and Large Intestines in Male and Female Mice in the Three-month Drinking Water Study of Sodium Metavanadate

	0 mg/L	31.3 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L
Male						
n[a]	10	0	10	10	10	10
Intestine, Small, Ileum						
Epithelium, hyperplasia[b]	0**	–[c]	0	2 (1.0)[d]	5* (2.0)	7** (1.3)
Intestine, Large, Cecum						
Erosion	0	–	–	–	–	1 (2.0)

	0 mg/L	31.3 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L
Intestine, Large, Colon						
Epithelium, hyperplasia	0	–	–	–	0	1 (1.0)
Intestine, Any Site	10					
Epithelium, hyperplasia	0**	–	0	2	5*	7**
Female						
n	10	0	10	10	10	10
Intestine, Small, Ileum						
Epithelium, hyperplasia	0**	–	0	2 (1.0)	9** (1.7)	8** (1.4)
Intestine, Small, Jejunum						
Epithelium, hyperplasia	0	–	–	–	0	1 (1.0)
Intestine, Any Site						
Epithelium, hyperplasia	0**	–	0	2	9**	8**

Statistical significance for an exposed 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]Number of animals examined microscopically.
[b]Number of animals with lesion. Statistical analyses performed by Cochran-Armitage (trend) and Fisher's exact (pairwise) one-sided tests.
[c]Data were not collected for an exposed group when animals exposed to higher concentrations exhibited no incidences of nonneoplastic lesions in that tissue.
[d]Average severity grade of observed lesion in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.

Kidney: In the kidneys of the male mice, renal tubule cytoplasmic alteration was seen in all animals in the 500 mg/L group and in two of the animals in the 250 mg/L group, and the severity increased with increasing exposure (Table 23). This lesion was not seen in the control group. This lesion was characterized by a decrease of the cytoplasmic vacuolation in relation to that of the control male mice.

Table 23. Incidences of Renal Tubule Cytoplasmic Alteration of the Kidney in Male Mice in the Three-month Drinking Water Study of Sodium Metavanadate

	0 mg/L	31.3 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L
Male						
Kidney[a]	10	0	10	10	10	10
Renal tubule, cytoplasmic alteration[b]	0**	–[c]	0	0	2 (2.0)[d]	10** (2.7)

Statistical significance for an exposed 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]Number of animals examined microscopically.
[b]Number of animals with lesion. Statistical analyses performed by Cochran-Armitage (trend) and Fisher's exact (pairwise) one-sided tests.
[c]Data were not collected for an exposed group when animals exposed to higher concentrations exhibited no incidences of nonneoplastic lesions in that tissue.
[d]Average severity grade of observed lesion in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.

Vanadyl Sulfate

Rats

Three-month Study (Perinatal Phase)

During gestation or lactation, there was no effect on survival of dams due to vanadyl sulfate exposure (Table 24). There were no effects in vanadyl sulfate-exposed dams as indicated by pregnancy and littering rates being similar across all groups.

Table 24. Summary of the Disposition of F_0 Female Rats during Perinatal Exposure in the Perinatal and Three-month Drinking Water Study of Vanadyl Sulfate

Reproductive Performance	0 mg/L	21.0 mg/L	41.9 mg/L	83.8 mg/L	168 mg/L	335 mg/L
Time-mated Females (GD 6)	16	16	16	16	16	16
Females Pregnant (%)[a]	15 (93.8)	16 (100)	16 (100)	16 (100)	15 (93.8)	16 (100)
Females Not Pregnant (%)	1 (6.2)	0 (0)	0 (0)	0 (0)	1 (6.2)	0 (0)
Pregnant Females Removed Prior to Littering (%)[b]	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (6.2)
Dams with Litters on LD 0 (%)[b]	15 (100)	16 (100)	16 (100)	16 (100)	15 (100)	15 (100)
Gestation Length (Days)[c,d]	21.9 ± 0.1 (15)	21.9 ± 0.1 (16)	22.1 ± 0.1 (16)	22.2 ± 0.1 (16)	21.8 ± 0.1 (15)	21.9 ± 0.1 (15)
Litters Poststandardization (PND 4)[e]	15	16	16	16	15	15
Litters at Weaning (LD 28)	15	16	15	14	15	15
F_1 Males/Females (PND 28)[f]	57/58	63/64	60/60	55/56	58/59	60/58

GD = gestation day; LD = lactation day; PND = postnatal day.
[a]Statistical analysis performed by the Cochran-Armitage (trend) and Fisher's exact (pairwise) tests.
[b]Percentage is given as a portion of pregnant dams.
[c]Statistical analysis performed by the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.
[d]Gestation length calculated for sperm-positive females that delivered a litter. Data are presented as mean ± standard error (number of dams).
[e]Standardization to eight pups/litter (four pups/sex).
[f]Number of males/females surviving until weaning on PND 28.

Gestational body weights of all exposed groups were within 10% of the control group, with a small but significant decrease in the 335 mg/L group (5%–6%) beginning on GD 18 (Table 25). Dam body weights during lactation were similar across all groups. There was a small but significant decrease on LD 1 in the 335 mg/L group (6%) that resolved by LD 4.

Table 25. Summary of 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 Vanadyl Sulfate

Parameter[a,b]	0 mg/L	21.0 mg/L	41.9 mg/L	83.8 mg/L	168 mg/L	335 mg/L
Gestation Body Weight						
Gestation Day						
6	245.5 ± 3.2 (15)	244.8 ± 3.0 (16)	244.7 ± 2.5 (16)	242.7 ± 3.1 (16)	243.3 ± 2.3 (15)	242.8± 3.1 (16)
9	258.7 ± 3.5 (15)	259.2 ± 3.2 (16)	259.1 ± 3.1 (16)	257.5 ± 3.2 (16)	257.3 ± 2.4 (15)	253.6 ± 3.9 (16)
12	273.7 ± 3.9* (15)	273.2 ± 3.3 (16)	273.2 ± 3.6 (16)	270.9 ± 3.3 (16)	269.7 ± 2.8 (15)	264.7 ± 3.6 (16)
15	293.0 ± 3.7* (15)	294.4 ± 3.8 (16)	293.1 ± 4.2 (16)	289.2 ± 3.7 (16)	288.6 ± 2.9 (15)	283.4 ± 4.2 (16)
18	334.3 ± 4.2** (15)	337.1 ± 4.9 (16)	334.4 ± 5.8 (16)	327.6 ± 4.9 (16)	325.2 ± 4.4 (15)	318.8 ± 5.2* (16)
21	386.1 ± 4.6** (15)	388.4 ± 5.7 (16)	387.5 ± 8.2 (16)	371.9 ± 5.8 (16)	372.9 ± 6.7 (15)	361.8 ± 7.6* (16)
Gestation Weight Change						
Gestation Day Interval						
6–9	13.1 ± 0.8 (15)	14.4 ± 0.6 (16)	14.4 ± 1.0 (16)	14.8 ± 0.7 (16)	14.1 ± 0.8 (15)	10.9 ± 1.5 (16)
9–12	15.0 ± 0.8** (15)	14.0 ± 0.9 (16)	14.1 ± 0.9 (16)	13.4 ± 0.7 (16)	12.3 ± 1.3 (15)	11.1 ± 0.7** (16)
12–15	19.3 ± 0.7 (15)	21.2 ± 1.0 (16)	19.9 ± 1.7 (16)	18.3 ± 1.1 (16)	18.9 ± 0.9 (15)	18.6 ± 0.8 (16)
15–18	41.3 ± 1.5** (15)	42.7 ± 1.6 (16)	41.3 ± 2.2 (16)	38.4 ± 2.3 (16)	36.6 ± 2.0 (15)	35.4 ± 1.7* (16)
18–21	51.8 ± 1.3* (15)	51.3 ± 1.5 (16)	53.1 ± 2.9 (16)	44.4 ± 2.4 (16)	47.7 ± 2.8 (15)	43.0 ± 3.8 (16)
6–21	140.6 ± 2.3** (15)	143.5 ± 4.0 (16)	142.7 ± 6.8 (16)	129.2 ± 4.3 (16)	129.6 ± 5.9 (15)	119.0 ± 5.7** (16)
Lactation Body Weight						
Lactation Day						
1	283.2 ± 4.4** (15)	285.5 ± 4.3 (16)	281.1 ± 3.8 (16)	273.0 ± 5.0 (16)	273.9 ± 3.0 (15)	267.2 ± 4.9* (15)
4	290.4 ± 4.6* (15)	290.5 ± 3.8 (16)	291.0 ± 4.5 (16)	283.3 ± 4.2 (16)	284.3 ± 3.6 (15)	277.3 ± 5.1 (15)
7	299.7 ± 4.3* (15)	300.4 ± 3.3 (16)	298.7 ± 4.7 (15)	294.7 ± 4.5 (15)	294.4 ± 4.0 (15)	287.6 ± 5.2 (15)
10	306.2 ± 4.1 (15)	306.1 ± 3.3 (16)	306.3 ± 4.9 (15)	307.3 ± 4.4 (15)	303.4 ± 3.9 (15)	299.8 ± 4.5 (15)
13	313.0 ± 4.1 (15)	306.5 ± 3.4 (16)	310.2 ± 4.6 (15)	310.8 ± 4.9 (15)	304.2 ± 4.6 (15)	303.8 ± 4.7 (15)
16	316.8 ± 4.4 (15)	314.3 ± 3.6 (16)	320.8 ± 4.4 (15)	313.1 ± 4.6 (15)	314.6 ± 5.2 (15)	304.5 ± 5.3 (15)
19	306.0 ± 4.3 (15)	307.3 ± 4.0 (16)	306.9 ± 3.8 (15)	308.5 ± 4.3 (15)	307.3 ± 4.0 (15)	301.0 ± 4.3 (15)
21	300.5 ± 4.4 (15)	304.6 ± 4.6 (16)	306.3 ± 4.8 (15)	302.6 ± 5.0 (14)	301.5 ± 4.1 (15)	296.1 ± 5.9 (15)
25	282.9 ± 5.7 (15)	279.7 ± 3.8 (16)	282.3 ± 4.4 (15)	288.1 ± 4.4 (14)	286.3 ± 4.1 (15)	281.1 ± 4.6 (15)
28	285.3 ± 4.9 (15)	278.0 ± 4.0 (16)	282.3 ± 4.2 (15)	285.0 ± 4.3 (14)	285.7 ± 3.7 (15)	280.8 ± 4.9 (15)
Lactation Weight Change						
Lactation Day Interval						
1–4	7.2 ± 1.6 (15)	5.0 ± 3.5 (16)	9.9 ± 2.0 (16)	10.3 ± 2.0 (16)	10.3 ± 1.9 (15)	10.1 ± 1.8 (15)
4–7	9.2 ± 1.5 (15)	9.9 ± 1.4 (16)	7.5 ± 1.7 (15)	9.9 ± 2.4 (15)	10.1 ± 2.2 (15)	10.3 ± 2.2 (15)
7–10	6.5 ± 2.3* (15)	5.7 ± 2.5 (16)	7.6 ± 2.2 (15)	12.6 ± 2.8 (15)	9.0 ± 2.3 (15)	12.2 ± 2.3 (15)
10–13	6.8 ± 2.2 (15)	0.4 ± 2.8 (16)	3.9 ± 2.2 (15)	3.5 ± 2.1 (15)	0.7 ± 2.4 (15)	4.1 ± 3.1 (15)
13–16	3.8 ± 1.7 (15)	7.8 ± 2.3 (16)	10.6 ± 2.1 (15)	2.3 ± 2.4 (15)	10.4 ± 2.7 (15)	0.7 ± 3.6 (15)
16–19	−10.8 ± 1.9 (15)	−7.0 ± 1.5 (16)	−13.9 ± 2.2 (15)	−4.5 ± 2.6 (15)	−7.3 ± 2.7 (15)	−3.5 ± 3.4 (15)
19–21	−5.5 ± 2.0 (15)	−2.7 ± 2.2 (16)	−0.6 ± 3.1 (15)	−4.5 ± 2.1 (14)	−5.8 ± 2.3 (15)	−4.9 ± 3.0 (15)
21–25	−17.6 ± 2.9 (15)	−24.8 ± 2.2 (16)	−24.0 ± 2.8 (15)	−14.5 ± 2.8 (14)	−15.2 ± 2.8 (15)	−15.0 ± 3.3 (15)
25–28	2.4 ± 1.9 (15)	−1.8 ± 1.1 (16)	0.0 ± 2.8 (15)	−3.1 ± 2.2 (14)	−0.6 ± 2.1 (15)	−0.3 ± 2.4 (15)
1–28	2.1 ± 2.7** (15)	−7.5 ± 3.9 (16)	0.5 ± 2.3 (15)	8.4 ± 3.1 (14)	11.8 ± 3.1* (15)	13.6 ± 2.3** (15)

Statistical significance for an exposed 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 exposed 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 are presented as mean ± standard error (number of dams). Body weight data are presented in grams.

Water consumption during gestation (GD 6–21) was significantly decreased at ≥83.8 mg/L in a concentration-dependent manner, with rats in the 335 mg/L group consuming 37% less water than the control group (Table 26). Water consumption during lactation (LD 1–13) significantly decreased by 11% in the 335 mg/L group compared to the control group; all other exposed groups were similar to the control group during lactation. Vanadyl sulfate intake during gestation (GD 6–21) and lactation (LD 1–13) are presented in Table 26

Table 26. Summary of Water and Vanadyl Sulfate Consumption by F_0 Female Rats during Gestation and Lactation in the Perinatal and Three-month Drinking Water Study

Parameter[a]	0 mg/L	21.0 mg/L	41.9 mg/L	83.8 mg/L	168 mg/L	335 mg/L
Gestation Day Interval (g/kg/day)[b]						
6–9	143.3 ± 7.0** (15)	129.6 ± 4.0 (16)	131.8 ± 3.2 (16)	125.6 ± 4.5 (16)	105.4 ± 3.1** (14)	87.5 ± 3.6** (16)
9–12	145.8 ± 6.6** (15)	134.6 ± 4.1 (16)	135.0 ± 4.5 (16)	129.2 ± 5.6* (16)	106.2 ± 3.1** (15)	90.2 ± 2.9** (16)
12–15	144.4 ± 8.2** (15)	126.0 ± 3.3 (16)	130.2 ± 4.1 (16)	118.2 ± 4.0** (16)	98.9 ± 3.5** (15)	82.2 ± 3.0** (16)
15–18	168.9 ± 7.2** (15)	153.5 ± 4.6 (16)	163.0 ± 7.0 (16)	143.2 ± 6.0** (16)	129.1 ± 4.2** (15)	112.6 ± 2.9** (16)
18–21	143.1 ± 5.8** (15)	128.4 ± 3.9 (16)	135.3 ± 6.4 (16)	120.1 ± 4.4** (16)	108.1 ± 4.2** (15)	96.6 ± 2.6** (16)
6–21	147.6 ± 6.4** (15)	133.0 ± 3.1 (16)	137.9 ± 4.8 (16)	126.0 ± 4.5** (16)	108.9 ± 3.1** (15)	93.5 ± 2.6** (16)
Lactation Day Interval (g/kg/day)[b]						
1–4	182.7 ± 7.0** (15)	174.0 ± 4.7 (16)	170.5 ± 6.0 (16)	163.1 ± 5.6* (16)	165.5 ± 5.1* (15)	157.0 ± 5.2** (15)
4–7	184.6 ± 6.6 (15)	187.0 ± 3.9 (16)	193.8 ± 5.7 (15)	193.5 ± 4.1 (15)	177.5 ± 5.3 (15)	171.6 ± 3.8 (15)
7–10	220.3 ± 8.6 (15)	215.2 ± 4.2 (16)	226.1 ± 6.4 (15)	231.7 ± 6.5 (15)	210.8 ± 5.8 (15)	200.2 ± 4.7 (15)
10–13	252.1 ± 9.3** (15)	251.8 ± 5.6 (16)	249.8 ± 7.3 (15)	247.7 ± 6.6 (15)	227.2 ± 8.3 (15)	214.9 ± 4.9** (15)
1–13	210.6 ± 7.4** (15)	208.0 ± 3.5 (16)	211.8 ± 5.7 (15)	211.1 ± 4.6 (15)	196.5 ± 5.5 (15)	187.3 ± 3.9** (15)
Chemical Intake (mg/kg/day)[c,d]						
GD 6–21	0.0 ± 0.0 (15)	2.8 ± 0.1 (16)	5.8 ± 0.2 (16)	10.6 ± 0.4 (16)	18.3 ± 0.5 (15)	31.3 ± 0.9 (16)
LD 1–13	0.0 ± 0.0 (15)	4.4 ± 0.1 (16)	8.9 ± 0.2 (15)	17.7 ± 0.4 (15)	33.0 ± 0.9 (15)	62.8 ± 1.3 (15)

Statistical significance for an exposed 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 are presented as mean ± standard error (number of dams).
[b]Each exposed 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.
[c]Chemical intake calculated as: ([exposure concentration × water consumption]/[average body weight of day range]).
[d]No statistical analysis was performed on the chemical intake data.

Vanadyl sulfate exposure did not significantly affect litter size or pup viability (Table 27). However, total and live litter sizes in the 335 mg/L group were 10% smaller than the control group at PND 0. There was a significant difference in live litter size on PND 0 in the 83.8 mg/L group, but this was considered a spurious finding attributable to a small increase in the number of dead pups on PND 0. Live litter size in the 168 mg/L group was similar to the control group, and therefore the lack of an exposure concentration-related response suggests the effects at 83.8 mg/L were not exposure concentration-related. Male and female pup body weights of vanadyl sulfate-exposed groups were within 5% of control groups throughout lactation (Table 28).

Table 27. Summary of Litter Size and Survival Ratio of F_1 Male and Female Rats during Lactation in the Perinatal and Three-month Drinking Water Study of Vanadyl Sulfate

Postnatal Day	0 mg/L	21.0 mg/L	41.9 mg/L	83.8 mg/L	168 mg/L	335 mg/L
Total Litter Size[a,b]						
0	14.5 ± 0.4 (15)	14.4 ± 0.5 (16)	13.9 ± 0.9 (16)	13.0 ± 0.7 (16)	13.7 ± 0.7 (15)	13.0 ± 0.8 (15)
Live Litter Size[a,b]						
0	14.2 ± 0.4 (15)	13.9 ± 0.5 (16)	13.1 ± 0.8 (16)	11.8 ± 0.8* (16)	13.3 ± 0.8 (15)	12.8 ± 0.7 (15)
1	14.2 ± 0.4 (15)	13.8 ± 0.5 (16)	12.9 ± 0.8 (16)	11.5 ± 0.9* (16)	13.1 ± 0.8 (15)	12.8 ± 0.7 (15)
4 (Prestandardization)	14.1 ± 0.4 (15)	13.8 ± 0.5 (16)	12.8 ± 0.7 (16)	11.4 ± 0.8* (16)	13.1 ± 0.8 (15)	12.7 ± 0.7 (15)
4 (Poststandardization)	8.0 ± 0.0 (15)	8.0 ± 0.0 (16)	7.8 ± 0.3 (16)	7.6 ± 0.3 (16)	7.8 ± 0.2 (15)	7.9 ± 0.1 (15)
7	7.9 ± 0.1 (15)	7.9 ± 0.1 (16)	8.0 ± 0.0 (15)[c]	7.9 ± 0.1 (15)[c]	7.8 ± 0.2 (15)	7.9 ± 0.1 (15)
10	7.8 ± 0.1 (15)	7.9 ± 0.1 (16)	8.0 ± 0.0 (15)	7.9 ± 0.1 (15)	7.8 ± 0.2 (15)	7.9 ± 0.1 (15)
13	7.7 ± 0.2 (15)	7.9 ± 0.1 (16)	8.0 ± 0.0 (15)	7.9 ± 0.1 (15)	7.8 ± 0.2 (15)	7.9 ± 0.1 (15)
16	7.7 ± 0.2 (15)	7.9 ± 0.1 (16)	8.0 ± 0.0 (15)	7.9 ± 0.1 (15)	7.8 ± 0.2 (15)	7.9 ± 0.1 (15)
19	7.7 ± 0.2 (15)	7.9 ± 0.1 (16)	8.0 ± 0.0 (15)	7.9 ± 0.1 (15)	7.8 ± 0.2 (15)	7.9 ± 0.1 (15)
21	7.7 ± 0.2 (15)	7.9 ± 0.1 (16)	8.0 ± 0.0 (15)	7.9 ± 0.1 (14)[c]	7.8 ± 0.2 (15)	7.9 ± 0.1 (15)
25	7.7 ± 0.2 (15)	7.9 ± 0.1 (16)	8.0 ± 0.0 (15)	7.9 ± 0.1 (14)	7.8 ± 0.2 (15)	7.9 ± 0.1 (15)
28	7.7 ± 0.2 (15)	7.9 ± 0.1 (16)	8.0 ± 0.0 (15)	7.9 ± 0.1 (14)	7.8 ± 0.2 (15)	7.9 ± 0.1 (15)
% Live Male per Litter[a,b,d]						
0	45.83 ± 3.23 (15)	50.20 ± 3.21 (16)	49.74 ± 4.86 (16)	45.06 ± 4.44 (16)	51.22 ± 4.41 (15)	50.19 ± 4.09 (15)
No. of Dead Pups (Litters)[e,f]						
0	4 (4)	8 (5)	13 (6)	20 (7)	7 (4)	3 (2)
1–4	1 (1)	1 (1)	5 (4)	5 (4)	2 (1)	1 (1)
5–28	5 (4)	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)

Postnatal Day	0 mg/L	21.0 mg/L	41.9 mg/L	83.8 mg/L	168 mg/L	335 mg/L
Dead per Litter[a,b,g]						
0	0.27 ± 0.12 (15)	0.50 ± 0.20 (16)	0.81 ± 0.34 (16)	1.25 ± 0.68 (16)	0.47 ± 0.27 (15)	0.20 ± 0.14 (15)
1–4	0.07 ± 0.07 (15)	0.06 ± 0.06 (16)	0.31 ± 0.15 (16)	0.31 ± 0.15 (16)	0.13 ± 0.13 (15)	0.07 ± 0.07 (15)
5–28	0.33 ± 0.16** (15)	0.06 ± 0.06 (16)	0.00 ± 0.00* (15)	0.00 ± 0.00* (14)	0.00 ± 0.00** (15)	0.00 ± 0.00** (15)
Survival Ratio[a,b]						
0[h]	0.98 ± 0.01 (15)	0.97 ± 0.01 (16)	0.95 ± 0.02 (16)	0.92 ± 0.04 (16)	0.97 ± 0.02 (15)	0.99 ± 0.01 (15)
1–4[i]	1.00 ± 0.00 (15)	0.99 ± 0.01 (16)	0.98 ± 0.01 (16)	0.96 ± 0.03 (16)	0.99 ± 0.01 (15)	1.00 ± 0.00 (15)
5–28[j]	0.96 ± 0.02** (15)	0.99 ± 0.01 (16)	1.00 ± 0.00* (15)	1.00 ± 0.00* (14)	1.00 ± 0.00** (15)	1.00 ± 0.00** (15)

Statistical significance for an exposed 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 are presented as mean of the litter means ± standard error (number of litters).
[b]Each exposed 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.
[c]There were three dam and respective litter removals between LD 4 (poststandardization) and LD 20: two dams and respective litters were removed during litter standardizations on LD 4 due to insufficient litter sizes from the 41.9 mg/L and 83.8 mg/L groups, and one dam was found dead on LD 20 in the 83.3 mg/L group.
[d]100 × [number of live males in exposure group]/[number of live males and females in exposure group](number of pups).
[e]Total number of dead pups in exposure group (number of litters contributing dead pups).
[f]No statistical analysis was performed on this endpoint.
[g]Number dead per litter (number of litters).
[h]Survival per litter: Number of live pups on PND 0/total number of pups upon completion of parturition.
[i]Survival per litter: Number of pups prestandardization on PND 4/total live pups on PND 0.
[j]Survival per litter: Number of live pups on PND 28/number of live pups poststandardization on PND 4.

Table 28. Summary of Preweaning F_1 Male and Female Rat Pup Body Weights Following Perinatal Exposure to Vanadyl Sulfate

Postnatal Day	0 mg/L	21.0 mg/L	41.9 mg/L	83.8 mg/L	168 mg/L	335 mg/L
Male						
1[a,b,c]	7.09 ± 0.21 (98/15)	7.07 ± 0.09 (110/16)	7.31 ± 0.10 (107/15)	7.00 ± 0.17 (86/15)	7.05 ± 0.16 (100/15)	7.06 ± 0.13 (96/15)
4[d,e,f,g]	9.93 ± 0.26 (97/15)	10.36 ± 0.15 (110/16)	10.44 ± 0.17 (107/15)	9.99 ± 0.22 (86/15)	10.15 ± 0.24 (100/15)	10.21 ± 0.19 (96/15)
7[d,e,h]	15.76 ± 0.50 (58/15)	16.96 ± 0.31 (63/16)	17.11 ± 0.44 (60/15)	17.09 ± 0.50 (59/15)	16.11 ± 0.42 (58/15)	16.46 ± 0.34 (60/15)
10[d,e,h]	23.33 ± 0.56 (58/15)	24.38 ± 0.38 (63/16)	24.67 ± 0.55 (60/15)	24.32 ± 0.65 (59/15)	23.70 ± 0.49 (58/15)	23.78 ± 0.48 (60/15)
13[d,e,h]	31.49 ± 0.53 (58/15)	32.15 ± 0.41 (63/16)	32.55 ± 0.56 (60/15)	32.24 ± 0.80 (59/15)	31.66 ± 0.59 (58/15)	31.27 ± 0.51 (60/15)
16[d,e,h]	38.88 ± 0.59 (58/15)	39.11 ± 0.53 (63/16)	39.24 ± 0.66 (60/15)	39.05 ± 0.99 (59/15)	38.27 ± 0.57 (58/15)	37.69 ± 0.59 (60/15)
19[d,e,h]	48.53 ± 0.90* (57/15)	49.61 ± 0.82 (63/16)	50.17 ± 1.02 (60/15)	49.17 ± 1.40 (59/15)	47.83 ± 0.92 (58/15)	46.90 ± 0.81 (60/15)
21[d,e,h]	57.44 ± 0.99** (57/15)	59.04 ± 0.83 (63/16)	59.53 ± 1.17 (60/15)	58.61 ± 1.18 (55/14)	56.37 ± 0.93 (58/15)	55.61 ± 0.93 (60/15)
25[d,e,h]	74.64 ± 1.21* (57/15)	77.25 ± 1.26 (63/16)	77.47 ± 1.32 (60/15)	76.98 ± 1.35 (55/14)	73.88 ± 1.18 (58/15)	73.75 ± 1.10 (60/15)
28[d,e,h]	92.26 ± 1.53** (57/15)	94.53 ± 1.32 (63/16)	95.50 ± 1.56 (60/15)	94.40 ± 1.38 (55/14)	89.32 ± 1.46 (58/15)	88.73 ± 1.21 (60/15)
Female						
1	6.52 ± 0.11 (115/15)	6.75 ± 0.11 (111/16)	6.87 ± 0.17 (100/16)	6.76 ± 0.21 (98/16)	6.70 ± 0.14 (97/15)	6.81 ± 0.16 (96/15)
4	9.24 ± 0.23 (115/15)	9.91 ± 0.19 (111/16)	9.95 ± 0.16 (97/16)	9.92 ± 0.29 (97/16)	9.57 ± 0.23 (97/15)	9.79 ± 0.21 (95/15)
7	14.42 ± 0.43 (60/15)	16.08 ± 0.35* (64/16)	16.30 ± 0.5** (60/15)	17.19 ± 0.37** (60/15)	15.19 ± 0.43 (59/15)	15.61 ± 0.40 (58/15)
10	21.44 ± 0.60 (59/15)	23.29 ± 0.49 (64/16)	23.59 ± 0.64* (60/15)	24.26 ± 0.47** (60/15)	22.52 ± 0.51 (59/15)	22.56 ± 0.56 (58/15)
13	29.35 ± 0.64 (58/15)	30.99 ± 0.61 (64/16)	31.25 ± 0.62 (60/15)	32.08 ± 0.68* (60/15)	30.40 ± 0.61 (59/15)	29.84 ± 0.58 (58/15)
16	36.49 ± 0.68 (58/15)	37.57 ± 0.73 (64/16)	37.78 ± 0.71 (60/15)	38.68 ± 0.88 (60/15)	36.91 ± 0.60 (59/15)	35.94 ± 0.63 (58/15)
19	45.47 ± 0.87* (58/15)	47.65 ± 1.03 (64/16)	47.49 ± 0.94 (60/15)	48.37 ± 1.06 (60/15)	45.73 ± 0.91 (59/15)	44.67 ± 0.92 (58/15)
21	53.34 ± 0.98* (58/15)	55.80 ± 1.06 (64/16)	56.05 ± 1.03 (60/15)	56.93 ± 0.73 (56/14)	53.47 ± 0.99 (59/15)	52.27 ± 0.99 (58/15)
25	68.06 ± 1.14 (58/15)	71.31 ± 1.36 (64/16)	71.73 ± 1.22 (60/15)	72.95 ± 0.93* (56/14)	68.64 ± 1.13 (59/15)	68.13 ± 1.11 (58/15)
28	82.88 ± 1.52** (58/15)	85.24 ± 1.45 (64/16)	86.19 ±1.46 (60/15)	86.87 ± 0.76 (56/14)	81.91 ± 1.17 (59/15)	80.18 ± 1.07 (58/15)

Postnatal Day	0 mg/L	21.0 mg/L	41.9 mg/L	83.8 mg/L	168 mg/L	335 mg/L
Male and Female						
1	6.77 ± 0.15 (213/15)	6.91 ± 0.08 (221/16)	7.06 ± 0.13 (207/16)	6.85 ± 0.21 (184/16)	6.90 ± 0.15 (197/15)	6.96 ± 0.14 (192/15)
4	9.57 ± 0.23 (212/15)	10.15 ± 0.15 (221/16)	10.15 ± 0.17 (204/16)	9.88 ± 0.29 (183/16)	9.90 ± 0.23 (197/15)	10.00 ± 0.19 (191/15)
7	15.08 ± 0.46 (118/15)	16.52 ± 0.30* (127/16)	16.70 ± 0.45* (120/15)	17.19 ± 0.40** (119/15)	15.64 ± 0.42 (117/15)	16.05 ± 0.34 (118/15)
10	22.38 ± 0.57 (117/15)	23.83 ± 0.39 (127/16)	24.13 ± 0.57 (120/15)	24.35 ± 0.51* (119/15)	23.11 ± 0.49 (117/15)	23.19 ± 0.49 (118/15)
13	30.43 ± 0.57 (116/15)	31.57 ± 0.46 (127/16)	31.91 ± 0.55 (120/15)	32.22 ± 0.71 (119/15)	31.01 ± 0.59 (117/15)	30.59 ± 0.50 (118/15)
16	37.67 ± 0.61 (116/15)	38.33 ± 0.57 (127/16)	38.51 ± 0.64 (120/15)	38.93 ± 0.91 (119/15)	37.59 ± 0.56 (117/15)	36.84 ± 0.57 (118/15)
19	46.97 ± 0.86* (115/15)	48.62 ± 0.85 (127/16)	48.84 ± 0.93 (120/15)	48.85 ± 1.18 (119/15)	46.78 ± 0.90 (117/15)	45.82 ± 0.78 (118/15)
21	55.36 ± 0.96** (115/15)	57.41 ± 0.86 (127/16)	57.79 ± 1.02 (120/15)	57.89 ± 0.88 (111/14)	54.89 ± 0.94 (117/15)	53.99 ± 0.89 (118/15)
25	71.32 ± 1.13* (115/15)	74.28 ± 1.21 (127/16)	74.60 ± 1.15 (120/15)	75.10 ± 1.03 (111/14)	71.22 ± 1.13 (117/15)	71.02 ± 1.01 (118/15)
28	87.55 ± 1.49** (115/15)	89.88 ± 1.25 (127/16)	90.85 ± 1.43 (120/15)	90.80 ± 0.92 (111/14)	85.52 ± 1.30 (117/50)	84.57 ± 1.04 (118/15)

Statistical significance for an exposed 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 are presented as mean ± standard error (number of pups/number of litters). Body weight data are presented in grams.
[b]Each exposed 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]Total pup weight on postnatal day (PND) 1 divided by number of live pups on PND 1.
[d]Data are presented as mean of the litter means ± standard error (number of pups/number of litters). Body weight data are presented in grams.
[e]Statistical analysis performed using mixed models with random litter effect for both trend and pairwise tests, using the Dunnett-Hsu adjustment for multiple comparisons.
[f]PND 4 prestandardization.
[g]Individual pup weights first adjusted for live litter size on PND 1.
[h]Individual pup weights first adjusted for live litter size on PND 4 poststandardization.

Three-month Study (Postweaning Phase)

All male and female rats survived until the end of the study, and terminal body weights of the exposed groups were approximately equal to the control groups (Table 29, Table 30; Figure 4).

Table 29. Summary of Survival and Body Weights of Male Rats in the Perinatal and Three-month Drinking Water Study of Vanadyl Sulfate

Postnatal Day	0 mg/L		21.0 mg/L			41.9 mg/L			83.8 mg/L			168 mg/L			335 mg/L		
	Wt. (g)[a,b]	n[c]	Wt. (g)	Wt. (% of Controls)	n	Wt. (g)	Wt. (% of Controls)	n	Wt. (g)	Wt. (% of Controls)	n	Wt. (g)	Wt. (% of Controls)	n	Wt. (g)	Wt. (% of Controls)	n
28[d]	91.8 ± 2.0	15 (12)	94.1 ± 1.4	102.5	15 (14)	92.4 ± 2.4	100.6	15 (11)	93.5 ± 2.5	101.9	14 (11)	89.6 ± 1.5	97.5	15 (12)	89.9 ± 1.3	97.9	15 (13)
35	131.2 ± 3.3*	15 (12)	129.3 ± 2.0	98.6	15 (14)	132.4 ± 3.0	100.9	15 (11)	131.2 ± 4.8	100.0	14 (11)	129.0 ± 1.8	98.3	15 (12)	122.9 ± 2.0	93.7	15 (13)
42	177.1 ± 4.6*	15 (12)	174.8 ± 3.4	98.7	15 (14)	179.6 ± 4.4	101.4	15 (11)	176.9 ± 5.6	99.9	14 (11)	174.7 ± 2.3	98.6	15 (12)	165.7 ± 2.9	93.6	15 (13)
49	224.5 ± 5.4**	15 (12)	226.1 ± 4.0	100.7	15 (14)	229.1 ± 4.6	102.0	15 (11)	226.1 ± 6.9	100.7	14 (11)	221.1 ± 2.5	98.5	15 (12)	209.4 ± 3.8	93.3	15 (13)
56	267.7 ± 6.5**	15 (12)	271.9 ± 4.5	101.6	15 (14)	275.6 ± 4.9	103.0	15 (11)	271.8 ± 7.1	101.5	14 (11)	264.6 ± 3.4	98.8	15 (12)	252.5 ± 3.8	94.3	15 (13)
63	310.3 ± 6.8**	15 (12)	313.9 ± 5.1	101.2	15 (14)	316.5 ± 5.5	102.0	15 (11)	311.9 ± 7.8	100.5	14 (11)	304.6 ± 4.0	98.2	15 (12)	294.9 ± 3.8	95.0	15 (13)
70	342.3 ± 7.8**	15 (12)	346.7 ± 5.3	101.3	15 (14)	346.7 ± 5.5	101.3	15 (11)	343.8 ± 8.1	100.5	14 (11)	330.7 ± 4.6	96.6	15 (12)	321.8 ± 4.9	94.0	15 (13)
77	365.7 ± 8.9**	15 (12)	371.2 ± 5.9	101.5	15 (14)	371.2 ± 5.9	101.5	15 (11)	368.3 ± 8.6	100.7	14 (11)	354.9 ± 5.9	97.0	15 (12)	347.0 ± 5.1	94.9	15 (13)
84	386.9 ± 9.3**	15 (12)	392.5 ± 7.1	101.4	15 (14)	390.2 ± 6.6	100.8	15 (11)	386.1 ± 8.9	99.8	14 (11)	373.0 ± 6.4	96.4	15 (12)	364.2 ± 5.5	94.1	15 (13)
91	400.8 ± 10.5**	15 (12)	410.7 ± 7.7	102.5	15 (14)	407.2 ± 7.0	101.6	15 (11)	402.7 ± 9.4	100.5	14 (11)	388.3 ± 6.4	96.9	15 (12)	377.2 ± 6.1	94.1	15 (13)
98	416.3 ± 10.7*	15 (12)	419.2 ± 9.0	100.7	15 (14)	422.3 ± 7.9	101.4	15 (11)	418.9 ± 9.5	100.6	14 (11)	403.7 ± 6.7	97.0	15 (12)	397.3 ± 5.5	95.4	15 (13)
105	426.3 ± 11.7*	15 (12)	442.2 ± 9.1	103.7	15 (14)	432.5 ± 8.0	101.5	15 (11)	434.9 ± 10.5	102.0	13 (10)	415.1 ± 7.4	97.4	15 (12)	406.1 ± 5.4	95.3	15 (13)
112	438.6 ± 11.5**	15 (12)	454.4 ± 8.0	103.6	15 (14)	442.9 ± 8.6	101.0	15 (11)	440.4 ± 11.4	100.4	14 (11)	415.2 ± 9.7	94.7	15 (12)	415.9 ± 6.2	94.8	15 (13)

Statistical significance for an exposed 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]Statistical analysis performed using mixed models with random litter effect for both trend and pairwise tests, using the Dunnett-Hsu adjustment for multiple comparisons.

[b]Weights shown are mean of the litter means ± standard error.

[c]Number of individual animals (number of litters); includes F_1 core and investigative study animals.

[d]Postnatal day 28 is the day animals were placed on study after pups were weaned.

Table 30. Summary of Survival and Body Weights of Female Rats in the Perinatal and Three-month Drinking Water Study of Vanadyl Sulfate

Postnatal Day	0 mg/L		21.0 mg/L			41.9 mg/L			83.8 mg/L			168 mg/L			335 mg/L		
	Wt. (g)[a,b]	n[c]	Wt. (g)	Wt. (% of Controls)	n	Wt. (g)	Wt. (% of Controls)	n	Wt. (g)	Wt. (% of Controls)	n	Wt. (g)	Wt. (% of Controls)	n	Wt. (g)	Wt. (% of Controls)	n
28[d]	84.2 ± 2.1*	15 (12)	85.0 ± 1.5	101.0	15 (14)	86.1 ± 2.8	102.3	15 (11)	87.4 ± 1.3	103.9	14 (11)	82.4 ± 1.8	98.0	15 (12)	80.5 ± 1.4	95.7	15 (13)
35	113.3 ± 2.7**	15 (12)	114.3 ± 2.1	100.9	15 (14)	117.6 ± 3.0	103.8	15 (11)	114.7 ± 1.7	101.2	14 (11)	111.5 ± 2.0	98.4	15 (12)	104.3 ± 1.8*	92.1	15 (13)
42	142.6 ± 3.6**	15 (12)	145.4 ± 2.7	101.9	15 (14)	151.0 ± 3.7	105.9	15 (11)	147.5 ± 3.1	103.4	14 (11)	145.0 ± 2.2	101.7	15 (12)	134.5 ± 2.3	94.3	15 (13)
49	172.8 ± 3.8**	15 (12)	171.4 ± 3.1	99.2	15 (14)	176.2 ± 4.0	102.0	15 (11)	173.1 ± 3.5	100.2	14 (11)	172.2 ± 2.9	99.7	15 (12)	162.0 ± 2.3	93.8	15 (13)
56	192.3 ± 4.2*	15 (12)	195.3 ± 3.8	101.6	15 (14)	196.7 ± 4.5	102.3	15 (11)	193.7 ± 4.0	100.7	14 (11)	195.1 ± 3.7	101.4	15 (12)	182.5 ± 1.8	94.9	15 (13)
63	212.8 ± 4.8	15 (12)	210.3 ± 3.9	98.8	15 (14)	216.0 ± 4.9	101.5	15 (11)	214.0 ± 3.8	100.5	14 (11)	214.6 ± 4.2	100.9	15 (12)	204.2 ± 3.1	95.9	15 (13)
70	224.2 ± 4.8	15 (12)	225.7 ± 4.8	100.7	15 (14)	230.3 ± 5.1	102.7	15 (11)	225.1 ± 5.1	100.4	14 (11)	228.5 ± 4.6	101.9	15 (12)	215.8 ± 2.2	96.3	15 (13)
77	239.6 ± 6.9	15 (12)	237.3 ± 4.5	99.0	15 (14)	243.8 ± 6.6	101.8	15 (11)	238.2 ± 4.8	99.4	14 (11)	241.0 ± 5.4	100.6	15 (12)	230.8 ± 2.7	96.3	15 (13)
84	248.4 ± 5.7	15 (12)	247.5 ± 5.2	99.6	15 (14)	254.3 ± 6.2	102.4	15 (11)	252.4 ± 4.8	101.6	14 (11)	253.0 ± 7.2	101.8	15 (12)	237.6 ± 3.7	95.6	15 (13)
91	251.4 ± 5.7	15 (12)	258.4 ± 6.0	102.8	15 (14)	262.1 ± 6.8	104.2	15 (11)	255.4 ± 5.5	101.6	14 (11)	259.0 ± 6.1	103.0	15 (12)	249.3 ± 4.3	99.2	15 (13)
98	263.6 ± 6.3	15 (12)	262.7 ± 4.8	99.7	15 (14)	269.0 ± 6.5	102.0	15 (11)	260.4 ± 6.0	98.8	14 (11)	265.1 ± 5.1	100.6	15 (12)	255.4 ± 4.0	96.9	15 (13)
105	267.4 ± 7.8	15 (12)	265.1 ± 5.1	99.1	15 (14)	272.9 ± 7.2	102.0	15 (11)	266.6 ± 5.1	99.7	14 (11)	272.0 ± 6.6	101.7	15 (12)	261.1 ± 3.0	97.7	15 (13)
112	267.6 ± 6.4	15 (12)	272.6 ± 4.7	101.9	15 (14)	279.9 ± 7.5	104.6	15 (11)	272.2 ± 5.6	101.7	14 (11)	277.1 ± 6.6	103.6	15 (12)	265.3 ± 3.7	99.2	15 (13)

Statistical significance for an exposed 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]Statistical analysis performed using mixed models with random litter effect for both trend and pairwise tests, using the Dunnett-Hsu adjustment for multiple comparisons.

[b]Weights shown are mean of the litter means ± standard error.

[c]Number of individual animals (number of litters); includes F_1 core and investigative study animals.

[d]Postnatal day 28 is the day animals were placed on study after pups were weaned.

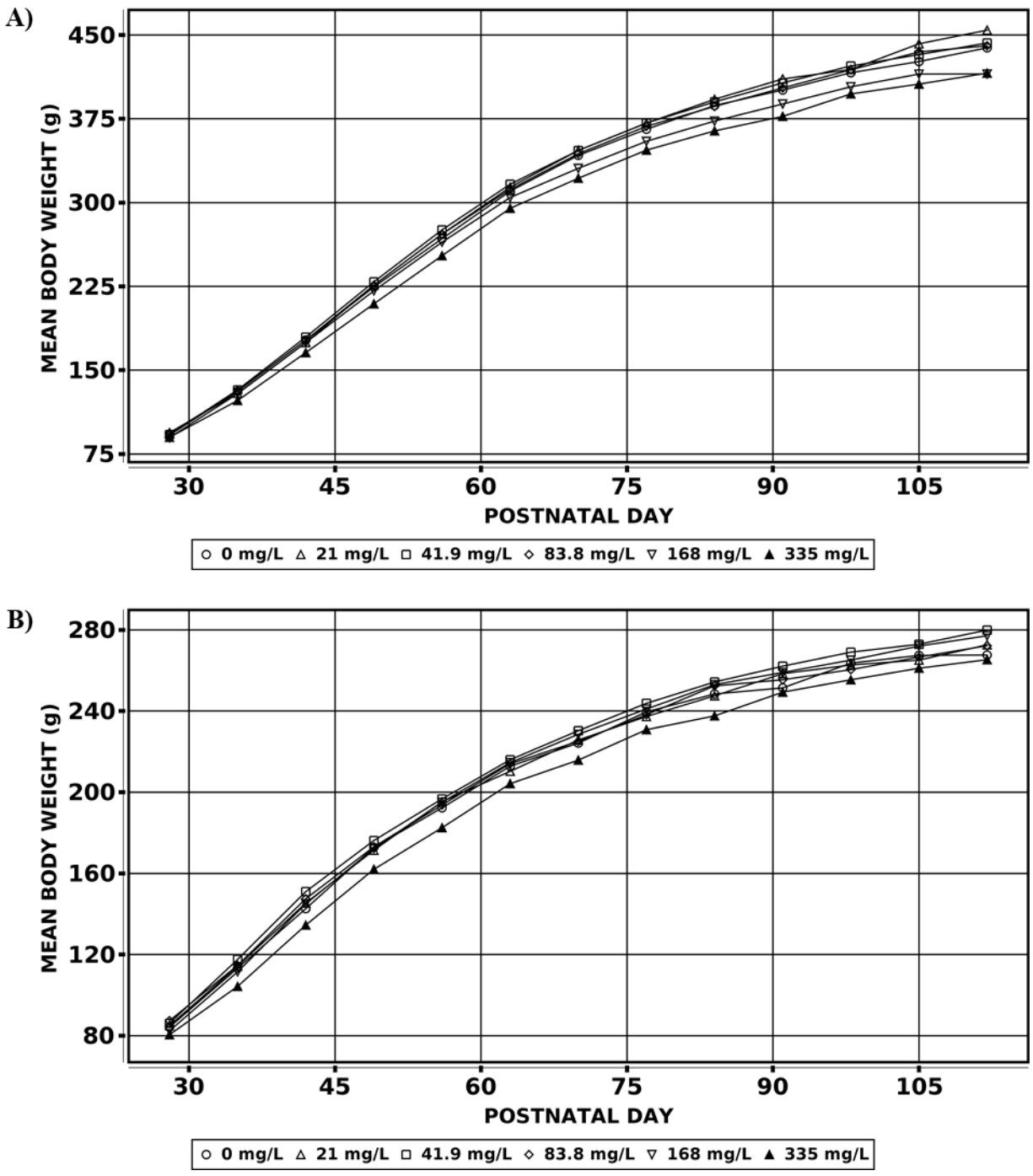


Figure 4. Growth Curves for Male and Female Rats in the Perinatal and Three-month Drinking Water Study of Vanadyl Sulfate

Growth curves are shown for (A) males and (B) females.

Water consumption was reduced in a concentration-dependent manner during the postweaning phase (PND 28–112) (Table 31). In male rats, there were significant decreases in water consumption by the 83.8 (10%), 168 (13%), and 335 (21%) mg/L groups for the duration of PND 28–112. In female rats, there was a significant decrease in water consumption over the PND 28–112 interval in the 335 (24%) mg/L group. Vanadyl sulfate intake over the 3-month postweaning phase (PND 28–112) is presented in Table 31.

Table 31. Summary of Water and Vanadyl Sulfate Consumption by Male and Female Rats in the Perinatal and Three-month Drinking Water Study

Postnatal Day[a]	0 mg/L	21.0 mg/L	41.9 mg/L	83.8 mg/L	168 mg/L	335 mg/L
Male (g/kg/day)[b]						
28–35	168.1 ± 7.7 (9)	151.5 ± 9.4 (9)	163.2 ± 6.1 (10)	160.6 ± 10.4 (9)	162.3 ± 3.0 (11)	143.4 ± 3.0* (9)
49–56	137.5 ± 4.2** (9)	132.4 ± 5.1 (9)	129.5 ± 3.9 (10)	123.5 ± 3.7* (10)	118.1 ± 2.8** (11)	112.4 ± 2.7** (9)
70–77	86.0 ± 2.0** (9)	83.0 ± 2.1 (9)	80.6 ± 2.6 (10)	78.2 ± 2.4* (10)	72.6 ± 1.6** (11)	67.6 ± 1.3** (9)
105–112	63.0 ± 2.7** (9)	58.5 ± 1.9 (9)	57.9 ± 1.8 (10)	55.1 ± 2.0* (10)	52.0 ± 1.3** (11)	47.3 ± 0.5** (9)
28–112	95.2 ± 2.8** (9)	90.3 ± 2.8 (9)	89.0 ± 1.8 (10)	85.8 ± 2.8* (10)	82.7 ± 1.8** (11)	75.3 ± 0.8** (9)
Chemical Intake (mg/kg/day)[c,d]						
28–112	0.0 ± 0.0 (9)	1.9 ± 0.1 (9)	3.7 ± 0.1 (10)	7.2 ± 0.2 (10)	13.9 ± 0.3 (11)	25.2 ± 0.3 (9)
Female (g/kg/day)						
28–35	159.3 ± 10.4 (6)	158.9 ± 11.3 (6)	172.1 ± 7.7 (9)	172.2 ± 8.9 (8)	164.2 ± 6.9 (8)	150.3 ± 7.4 (7)
49–56	138.6 ± 11.5 (6)	122.1 ± 6.5 (6)	124.4 ± 4.8 (9)	125.7 ± 5.0 (8)	128.5 ± 5.7 (8)	115.8 ± 3.4 (7)
70–77	108.1 ± 10.1* (6)	93.0 ± 4.8 (6)	97.9 ± 3.1 (9)	100.4 ± 5.2 (8)	96.9 ± 3.2 (8)	78.7 ± 2.4** (7)
105–112	90.6 ± 10.6* (6)	72.8 ± 8.1 (6)	81.2 ± 4.1 (9)	87.6 ± 7.5 (8)	75.4 ± 4.1 (8)	61.2 ± 3.0* (7)
28–112	117.1 ± 10.9* (6)	98.9 ± 5.1 (6)	106.3 ± 3.1 (9)	109.8 ± 5.2 (8)	101.5 ± 3.6 (8)	89.1 ± 2.8* (7)
Chemical Intake (mg/kg/day)						
28–112	0.0 ± 0.0 (6)	2.1 ± 0.1 (6)	4.5 ± 0.1 (9)	9.2 ± 0.4 (8)	17.1 ± 0.6 (8)	29.9 ± 1.0 (7)

Statistical significance for an exposed 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 are presented as mean ± standard error (number of cages).
[b]Each exposed 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.
[c]Chemical intake calculated as: ([exposure concentration × water consumption]/[average body weight of day range]).
[d]No statistical analysis was performed on the chemical intake data.

Vanadyl sulfate exposure had no effect on time to attainment of BPS in male rats (Appendix E). There was a positive trend for the day of VO attainment when data were unadjusted or adjusted for body weight at weaning (Table 32). Although not statistically significant, VO was 1.8 days or 1.6 days later in the 335 mg/L group when unadjusted or adjusted for body weight at weaning, respectively, compared to the control group.

Table 32. Summary of Vaginal Opening of F_1 Female Rats Exposed to Vanadyl Sulfate in the Perinatal and Three-month Drinking Water Study

Parameter[a]	0 mg/L	21.0 mg/L	41.9 mg/L	83.8 mg/L	168 mg/L	335 mg/L
No. Examined[b]	15 (12)	15 (14)	15 (11)	14 (11)	15 (12)	15 (13)
No. Not Attaining[c]	0	0	0	0	0	0
Day of VO						
Litter mean[d,e]	37.7 ± 0.5**	37.3 ± 0.7	36.6 ± 0.5	36.7 ± 1.0	37.7 ± 0.6	39.5 ± 0.6
Adjusted litter mean[d,f,g]	37.7 ± 0.5*	37.3 ± 0.7	36.7 ± 0.5	36.8 ± 1.0	37.6 ± 0.6	39.3 ± 0.6
Body Weight at Attainment (g)[h]	125.2 ± 3.4	123.4 ± 3.6	124.3 ± 3.2	122.8 ± 4.6	124.7 ± 3.7	122.1 ± 1.8
Body Weight at Weaning (g)[h]	84.8 ± 2.2*	84.8 ± 1.6	85.6 ± 2.9	87.2 ± 1.2	82.6 ± 1.7	80.6 ± 1.4

Statistical significance for the vehicle control group indicates a significant trend test.
*Statistically significant at $p \leq 0.05$; **$p \leq 0.01$.
VO = vaginal opening.
[a]Data are displayed as mean ± standard error unless otherwise noted; values are based on litter means, not individual pup values.
[b]No. Examined = the number of pups examined (number of litters).
[c]No. Not Attaining = number of pups that survived to the end of the observation period without attaining VO.
[d]Summary statistics and mixed model results are presented for animals that attained during the observation period.
[e]Statistical analysis performed using mixed effects models with exposure concentration as a covariate, litter as a random effect for both trend and pairwise tests, and a Dunnett-Hsu adjustment for multiple pairwise comparisons.
[f]Statistical analysis performed using mixed effects models with exposure concentration and weaning weight as covariates, litter as a random effect for both trend and pairwise tests, and a Dunnett-Hsu adjustment for multiple pairwise comparisons.
[g]Adjusted based on body weight at weaning.
[h]Analysis of body weight at attainment and body weight at weaning for both linear trend and pairwise comparisons performed using mixed effects models with exposure concentration as a covariate, litter as a random effect, and a Dunnett-Hsu adjustment for multiple pairwise comparisons.

Several spurious significant organ weight changes were observed and were not considered related to vanadyl sulfate exposure (Appendix E).

Male rats in the 168 and 335 mg/L groups displayed a slight (8%) but significant increase in cauda epididymal weights (Appendix E). No vanadyl sulfate-related changes in sperm counts, sperm motility, or histopathology in male reproductive organs were observed. In females, there was no effect of vanadyl sulfate on the number of estrous cycles or cycle length. Time in proestrus was lower in the 168 and 335 mg/L groups compared to the control group. Markov model estimates of stage length indicated that the length of proestrus was significantly decreased in the 335 mg/L group (Appendix E).

There were no hematology changes at PND 28 in F_1 rats following vanadyl sulfate exposure (Appendix E). There were no changes in hematology or clinical chemistry attributed to vanadyl sulfate exposure in rats at the end of the study (Appendix E).

Histopathology

This section describes the statistically significant or biologically noteworthy changes in the incidence of nonneoplastic lesions in the small and large intestines.

Small and large intestines: Epithelial hyperplasia was observed in the ileum of the small intestine and the cecum of the large intestine (Table 33). As with the sodium metavanadate studies, this lesion was most prevalent in the ileum of the small intestine. In males, this lesion was seen in the ileum in the ≥168 mg/L groups, with a positive trend and a significant pairwise comparison in the 335 mg/L group. The severity of this lesion increased slightly with increasing exposure concentration. Epithelial hyperplasia of the ileum was significantly increased in females in the ≥168 mg/L groups, with a positive trend. This lesion was also seen in the cecum in males and females in the 335 mg/L groups, with a positive trend and significant pairwise comparisons. Severity was comparable between males and females. Incidences of epithelial hyperplasia were observed at all intestinal sites (combined) in males and females exposed to ≥168 mg/L, with a positive trend and significant pairwise comparisons in the 335 mg/L male and ≥168 mg/L female groups. The lesion occurred in the ileum of all but one male and one female in the 335 mg/L groups. Epithelial hyperplasia was not observed in males or females exposed to ≤83.8 mg/L. The histological appearance of the epithelial hyperplasia was identical to that seen in rats exposed to sodium metavanadate (see Sodium Metavanadate Rat Histopathology). One male in the 335 mg/L group had a minimal amount of mineral in the wall of the jejunum. It is unclear whether this lesion was related to exposure.

Table 33. Incidences of Epithelial Hyperplasia of the Small and Large Intestines in Male and Female Rats in the Perinatal and Three-month Drinking Water Study of Vanadyl Sulfate

	0 mg/L	21.0 mg/L	41.9 mg/L	83.8 mg/L	168 mg/L	335 mg/L
Male						
n[a]	10	0	0	0[b]	10	10
Intestine, Small, Ileum						
Epithelium, hyperplasia[c]	0**	–[d]	–	–	3 (1.0)[e]	9** (1.2)
Intestine, Large, Cecum						
Epithelium, hyperplasia	0*	–	–	–	0	4* (1.0)
Intestine, Any Site						
Epithelium, hyperplasia	0**	–	–	0	3	9**
Female						
n	10	0	10	10	10	10
Intestine, Small, Ileum						
Epithelium, hyperplasia	0**	–	0	0	4* (1.3)	9** (1.4)
Intestine, Large, Cecum						
Epithelium, hyperplasia	0*	–	–	–	0	4* (1.0)
Intestine, Any Site						
Epithelium, hyperplasia	0**	–	0	0	4*	10**

Statistical significance for an exposed 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]Number of animals examined microscopically.
[b]For the intestine, any site data, n = 3 for the male 83.8 mg/L group.
[c]Number of animals with lesion. Statistical analyses performed by Cochran-Armitage (trend) and Fisher's exact (pairwise) one-sided tests.
[d]Data were not collected for an exposed group when animals exposed to higher concentrations exhibited no incidences of nonneoplastic lesions in that tissue.
[e]Average severity grade of observed lesion in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.

Mice

Three-month Study

There was no effect on survival of male or female mice due to vanadyl sulfate exposure (Table 34, Table 35; Figure 5). Significant decreases in body weight were consistently observed throughout the study in males in the 335 mg/L group, and terminal body weights were significantly decreased in the 168 mg/L group by 10% and in the 335 mg/L group by 13%. Although not significant, terminal body weight of the 335 mg/L females was slightly lower (9%) than that of the control group.

Table 34. Summary of Survival and Body Weights of Male Mice in the Three-month Drinking Water Study of Vanadyl Sulfate

Study Day[a]	0 mg/L		21.0 mg/L			41.9 mg/L			83.8 mg/L			168 mg/L			335 mg/L		
	Wt. (g)[b,c]	n	Wt. (g)	Wt. (% of Controls)	n	Wt. (g)	Wt. (% of Controls)	n	Wt. (g)	Wt. (% of Controls)	n	Wt. (g)	Wt. (% of Controls)	n	Wt. (g)	Wt. (% of Controls)	n
0	21.3 ± 0.3	10	21.3 ± 0.3	99.7	10	21.4 ± 0.4	100.1	10	21.4 ± 0.3	100.2	10	21.2 ± 0.4	99.5	10	21.4 ± 0.3	100.0	10
7	23.2 ± 0.3	10	23.1 ± 0.3	99.6	10	23.1 ± 0.4	99.7	10	23.1 ± 0.4	99.7	10	22.9 ± 0.3	98.8	10	22.4 ± 0.3	96.5	10
14	24.4 ± 0.3	10	24.4 ± 0.3	100.1	10	24.6 ± 0.4	100.9	10	24.3 ± 0.4	99.9	10	24.0 ± 0.4	98.6	10	23.3 ± 0.4	95.6	10
21	26.1 ± 0.4**	10	25.8 ± 0.4	99.0	10	25.9 ± 0.4	99.3	10	25.8 ± 0.5	99.0	10	25.3 ± 0.3	96.7	10	24.3 ± 0.4**	93.1	10
28	27.4 ± 0.5**	10	27.2 ± 0.4	99.1	10	27.0 ± 0.5	98.6	10	27.4 ± 0.6	99.9	10	26.3 ± 0.3	96.0	10	25.4 ± 0.4**	92.5	10
35	29.0 ± 0.6**	10	28.5 ± 0.5	98.4	10	28.8 ± 0.7	99.2	10	28.8 ± 0.7	99.4	10	27.5 ± 0.3	94.9	10	26.5 ± 0.4**	91.3	10
42	30.0 ± 0.7**	10	29.7 ± 0.6	98.8	10	30.0 ± 0.7	99.7	10	29.9 ± 0.8	99.6	10	28.2 ± 0.3	94.0	10	27.3 ± 0.4**	91.0	10
49	31.3 ± 0.7**	10	31.0 ± 0.7	99.1	10	31.1 ± 0.8	99.5	10	31.1 ± 0.8	99.3	10	29.3 ± 0.4	93.6	10	28.5 ± 0.5**	91.2	10
56	31.9 ± 0.8**	10	31.9 ± 0.8	99.9	10	31.9 ± 1.0	100.0	10	31.7 ± 0.8	99.4	10	29.7 ± 0.5	92.9	10	29.0 ± 0.6*	90.9	10
63	33.3 ± 1.0**	10	33.3 ± 0.9	100.1	10	33.5 ± 1.1	100.6	10	33.2 ± 0.9	99.8	10	30.7 ± 0.5*	92.1	10	29.9 ± 0.7**	89.8	10
70	34.0 ± 0.9**	10	34.2 ± 1.0	100.6	10	34.4 ± 1.2	101.4	10	33.3 ± 1.1	98.1	10	31.1 ± 0.6	91.5	10	29.9 ± 1.1**	87.9	10
77	35.0 ± 0.9**	10	35.7 ± 1.0	102.1	10	35.3 ± 1.2	100.8	10	35.0 ± 1.1	100.0	10	32.2 ± 0.7	92.0	10	31.1 ± 0.8**	88.8	10
84	35.8 ± 1.0**	10	36.1 ± 1.1	100.7	10	36.1 ± 1.4	100.7	10	35.9 ± 1.2	100.3	10	32.3 ± 0.8*	90.3	10	31.4 ± 0.8**	87.6	10
EOS[d]	37.2 ± 1.1**	10	37.0 ± 1.1	99.5	10	37.2 ± 1.4	100.0	10	37.2 ± 1.3	100.0	10	33.5 ± 0.8*	90.1	10	32.4 ± 0.9**	87.1	10

Statistical significance for an exposed 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 $p \leq 0.05$; **$p \leq 0.01$.
EOS = end of study.
[a]Study day 0 is the day animals were placed on study.
[b]Statistical analysis performed by the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.
[c]Weights shown are mean ± standard error.
[d]Half of the animals were euthanized on study day 91 and the other half on study day 92. Weights shown are the mean of all animals euthanized on study days 91 and 92.

Table 35. Summary of Survival and Body Weights of Female Mice in the Three-month Drinking Water Study of Vanadyl Sulfate

Study Day[a]	0 mg/L		21.0 mg/L			41.9 mg/L			83.8 mg/L			168 mg/L			335 mg/L		
	Wt. (g)[b,c]	n	Wt. (g)	Wt. (% of Controls)	n	Wt. (g)	Wt. (% of Controls)	n	Wt. (g)	Wt. (% of Controls)	n	Wt. (g)	Wt. (% of Controls)	n	Wt. (g)	Wt. (% of Controls)	n
0	17.0 ± 0.3	10	17.0 ± 0.4	99.9	10	17.4 ± 0.3	102.4	10	17.1 ± 0.3	101.0	10	17.4 ± 0.3	102.5	10	17.0 ± 0.4	100.3	10
7	18.4 ± 0.4	10	18.2 ± 0.5	99.0	10	18.5 ± 0.3	100.3	10	18.3 ± 0.3	99.2	10	18.7 ± 0.3	101.8	10	17.1 ± 0.5	92.9	10
14	19.6 ± 0.5	10	19.5 ± 0.4	99.6	10	19.5 ± 0.4	99.5	10	19.3 ± 0.3	98.4	10	19.6 ± 0.4	99.8	10	18.3 ± 0.4	93.4	10
21	20.7 ± 0.4	10	20.4 ± 0.5	98.8	10	20.5 ± 0.4	99.2	10	20.6 ± 0.5	99.5	10	20.6 ± 0.4	99.6	10	19.3 ± 0.4	93.3	10
28	21.5 ± 0.6	10	21.3 ± 0.5	99.1	10	21.7 ± 0.6	100.8	10	21.3 ± 0.6	99.2	10	21.5 ± 0.5	99.8	10	20.4 ± 0.3	94.7	10
35	22.8 ± 0.6	10	22.0 ± 0.5	96.6	10	21.9 ± 0.5	96.1	10	22.7 ± 0.8	99.6	10	22.6 ± 0.5	99.2	10	21.1 ± 0.4	92.7	10
42	23.5 ± 0.8	10	23.3 ± 0.6	99.4	10	22.5 ± 0.5	95.8	10	23.1 ± 0.8	98.5	10	23.3 ± 0.7	99.4	10	21.9 ± 0.4	93.2	10
49	24.3 ± 0.6	10	23.9 ± 0.7	98.3	10	24.1 ± 0.7	99.3	10	24.0 ± 0.9	98.8	10	25.0 ± 0.9	103.1	10	22.6 ± 0.4	93.0	10
56	25.1 ± 0.6	10	24.0 ± 0.8	95.8	10	23.9 ± 0.5	95.5	10	24.8 ± 1.0	99.1	10	25.1 ± 1.1	100.0	10	23.2 ± 0.5	92.7	10
63	25.8 ± 1.1	10	25.7 ± 0.8	99.6	10	26.0 ± 0.8	100.5	10	25.7 ± 1.1	99.3	10	26.4 ± 1.1	102.1	10	24.3 ± 0.6	93.8	10
70	26.8 ± 0.9	10	26.7 ± 0.8	99.3	10	26.9 ± 0.8	100.1	10	27.3 ± 1.3	101.6	10	27.7 ± 1.1	103.1	10	24.6 ± 0.5	91.5	10
77	27.3 ± 1.0	10	26.7 ± 1.0	97.6	10	27.4 ± 1.0	100.1	10	27.4 ± 1.3	100.2	10	27.7 ± 1.3	101.1	10	24.7 ± 0.5	90.5	10
84	27.2 ± 1.2	10	27.4 ± 1.1	100.9	10	27.6 ± 1.1	101.5	10	27.2 ± 1.2	100.1	10	28.2 ± 1.3	103.6	10	25.2 ± 0.6	92.6	10
91	27.7 ± 1.2	10	29.0 ± 1.1	104.6	10	29.2 ± 1.0	105.2	10	28.1 ± 1.2	101.4	10	28.8 ± 1.2	104.1	10	25.3 ± 0.6	91.4	10

[a]Study day 0 is the day animals were placed on study.
[b]Statistical analysis performed by the Jonckheere (trend) and Williams or Dunnett (pairwise) tests.
[c]Weights shown are mean ± standard error.

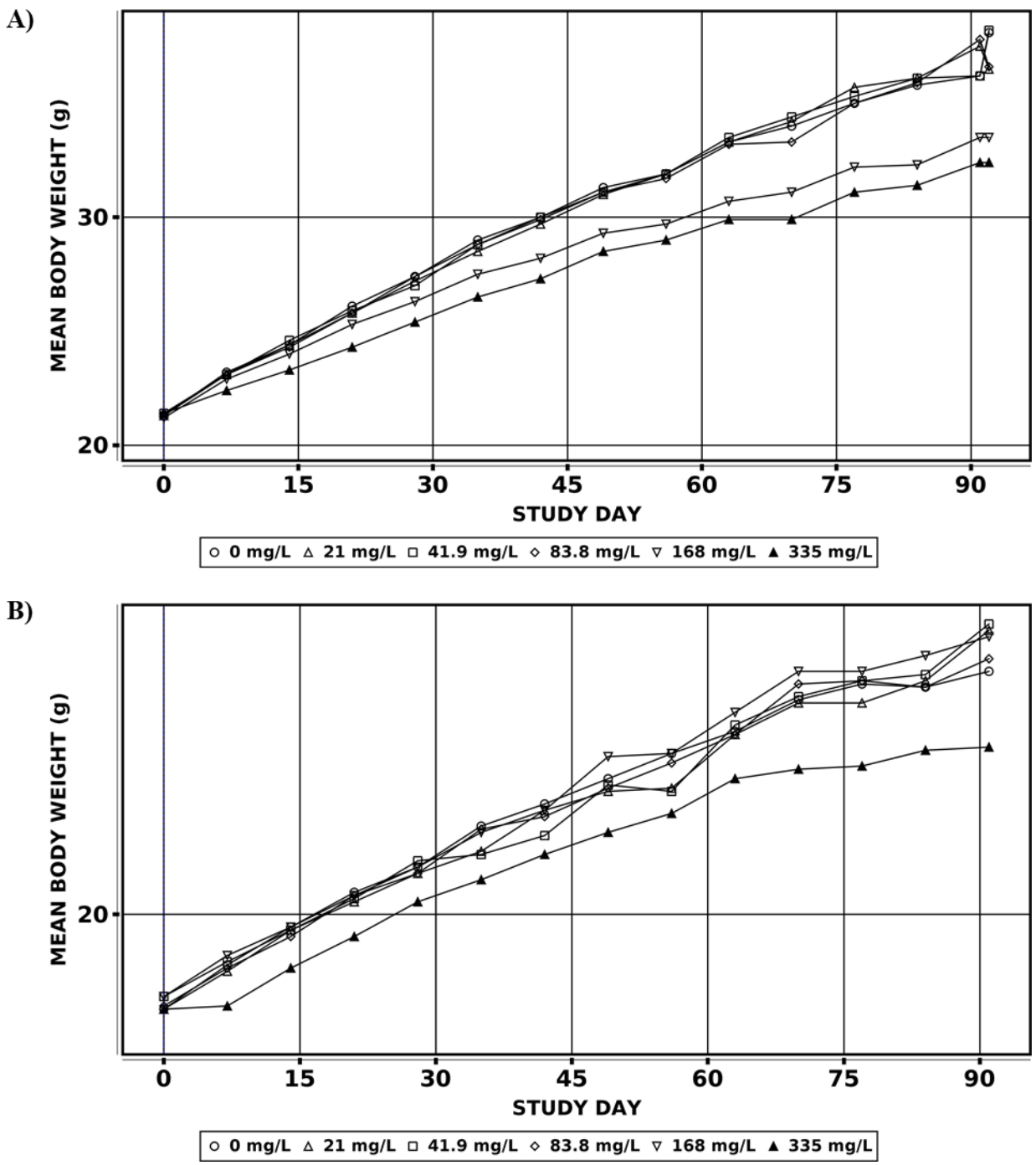


Figure 5. Growth Curves for Male and Female Mice in the Three-month Drinking Water Study of Vanadyl Sulfate

Growth curves are shown for (A) males and (B) females.

In males, water consumption over the study duration (study day 0–91) in the 335 mg/L group was 10% lower than the control group; all other exposed groups were similar to the control group. In females, water consumption over the study duration by all exposed groups was significantly decreased (10% to 17% lower than the control group) and exhibited a negative trend but was considered similar to the control group (i.e., no strong concentration response). Vanadyl sulfate intake over the course of the study (study day 0–91) is presented in Table 36.

Table 36. Summary of Water and Vanadyl Sulfate Consumption by Male and Female Mice in the Three-month Drinking Water Study

Study Day[a]	0 mg/L	21.0 mg/L	41.9 mg/L	83.8 mg/L	168 mg/L	335 mg/L
Male (g/kg/day)[b]						
0–7	143.8 ± 3.9** (10)	135.8 ± 4.0 (10)	141.0 ± 3.1 (10)	138.0 ± 3.9 (10)	139.4 ± 4.9 (10)	109.5 ± 3.3** (10)
21–28	129.1 ± 5.3* (10)	120.1 ± 2.8 (10)	131.0 ± 5.9 (10)	124.2 ± 2.6 (10)	125.7 ± 3.9 (10)	107.7 ± 2.5** (10)
84–91	98.7 ± 4.7 (10)	91.3 ± 4.1 (10)	96.8 ± 5.6 (10)	97.3 ± 4.1 (10)	107.5 ± 3.5 (10)	99.3 ± 3.3 (10)
0–91	114.5 ± 3.3 (10)	111.2 ± 3.2 (10)	115.1 ± 3.6 (10)	113.7 ± 3.1 (10)	119.0 ± 2.7 (10)	102.6 ± 2.5 (10)
Chemical Intake (mg/kg/day)[c,d]						
0–91	0.0 ± 0.0 (10)	2.3 ± 0.1 (10)	4.8 ± 0.2 (10)	9.5 ± 0.3 (10)	20.0 ± 0.5 (10)	34.4 ± 0.8 (10)
Female (g/kg/day)						
0–7	134.1 ± 3.7** (10)	110.7 ± 2.9** (10)	110.8 ± 2.3** (10)	114.2 ± 3.0** (10)	104.8 ± 4.8** (10)	71.7 ± 1.9** (10)
21–28	135.4 ± 2.9** (10)	118.6 ± 3.9** (10)	118.9 ± 3.0** (10)	105.7 ± 2.1** (10)	114.9 ± 2.1** (10)	100.2 ± 1.6** (10)
84–91	97.7 ± 4.2 (10)	91.0 ± 3.2 (10)	83.8 ± 3.2* (10)	91.9 ± 3.5 (10)	87.1 ± 3.7 (10)	95.0 ± 2.1 (10)
0–91	119.3 ± 3.4** (10)	107.4 ± 3.0* (10)	103.5 ± 2.8** (10)	99.6 ± 2.7** (10)	101.8 ± 2.8** (10)	102.9 ± 2.0** (10)
Chemical Intake (mg/kg/day)						
0–91	0.0 ± 0.0 (10)	2.3 ± 0.1 (10)	4.3 ± 0.1 (10)	8.3 ± 0.2 (10)	17.1 ± 0.5 (10)	34.5 ± 0.7 (10)

Statistical significance for an exposed 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 are presented as mean ± standard error (number of animals).
[b]Each exposed 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.
[c]Chemical intake calculated as: ([exposure concentration × water consumption]/[average body weight of day range]).
[d]No statistical analysis was performed on the chemical intake data.

Several significant relative organ weight changes were observed, but these were not considered toxicologically relevant (Appendix E).

Reticulocyte count was significantly increased in the 335 mg/L male group (Table 37). In both male and female mice, MCV was significantly decreased in the ≥168 mg/L groups; the decreases in MCV indicated microcytosis. Additionally, in male mice, hemoglobin was significantly decreased in the 335 mg/L group, MCH was significantly decreased in the ≥83.8 mg/L groups, and MCHC was significantly decreased in the ≥168 mg/L groups. In female mice, erythrocyte count was significantly increased in the ≥83.8 mg/L groups, MCH was significantly decreased in the ≥83.8 mg/L groups, and MCHC was significantly decreased in the ≥168 mg/L groups.

Table 37. Summary of Select Hematology Data for Male and Female Mice in the Three-month Drinking Water Study of Vanadyl Sulfate

Endpoint[a,b]	0 mg/L	21.0 mg/L	41.9 mg/L	83.8 mg/L	168 mg/L	335 mg/L
Male						
n	10	9	9	9	9	10
Hemoglobin (g/dL)	15.4 ± 0.2**	15.4 ± 0.1	15.4 ± 0.1	15.1 ± 0.1	15.0 ± 0.1	14.6 ± 0.1**
Erythrocytes (10^6/µL)	10.60 ± 0.12*	10.44 ± 0.19	10.68 ± 0.08	10.58 ± 0.12	10.79 ± 0.14	10.98 ± 0.10
Reticulocytes (10^3/µL)	265.8 ± 5.1**	264.2 ± 8.7	274.6 ± 9.4	276.8 ± 9.4	272.5 ± 8.7	302.0 ± 7.0**[c]
Mean Cell Volume (fL)	49.6 ± 0.2**	50.2 ± 0.3	49.5 ± 0.2	49.1 ± 0.2	48.6 ± 0.3*	47.0 ± 0.3**
Mean Cell Hemoglobin (pg)	14.5 ± 0.1**	14.8 ± 0.2	14.4 ± 0.1	14.3 ± 0.1*	13.9 ± 0.1**	13.3 ± 0.1**
Mean Cell Hemoglobin Concentration (g/dL)	29.3 ± 0.2**	29.5 ± 0.5	29.1 ± 0.1	29.0 ± 0.2	28.7 ± 0.1**	28.4 ± 0.2**
Female						
n	9	9	10	10	10	9
Hemoglobin (g/dL)	15.1 ± 0.1	15.0 ± 0.1	13.7 ± 1.3	15.3 ± 0.1	15.0 ± 0.2	14.4 ± 0.2
Erythrocytes (10^6/µL)	10.14 ± 0.09**	9.30 ± 0.98	9.28 ± 0.94	10.51 ± 0.06*	10.61 ± 0.12**	10.75 ± 0.13**
Reticulocytes (10^3/µL)	264.6 ± 21.6	261.7 ± 29.9	254.4 ± 32.5	303.2 ± 28.1	276.7 ± 11.5	251.4 ± 14.0
Mean Cell Volume (fL)	50.0 ± 0.3**	50.0 ± 0.2	49.4 ± 0.3	49.3 ± 0.2	48.3 ± 0.2**	46.7 ± 0.3**
Mean Cell Hemoglobin (pg)	14.9 ± 0.1**	23.7 ± 9.0	15.7 ± 1.0	14.6 ± 0.1*	14.1 ± 0.1**	13.4 ± 0.1**
Mean Cell Hemoglobin Concentration (g/dL)	29.8 ± 0.2**	47.7 ± 18.4	31.8 ± 2.0	29.6 ± 0.2	29.2 ± 0.1*	28.8 ± 0.1**

Statistical significance for an exposed 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 are presented as mean ± standard error.
[b]Statistical analysis performed by the Jonckheere (trend) and Shirley or Dunn (pairwise) tests.
[c]n = 9. One sample in the indicated group was excluded from analysis due to biological implausibility.

In male mice, there were no vanadyl sulfate-related changes in sperm counts or motility parameters (Appendix E). In female mice, there was no effect of vanadyl sulfate on the number of estrous cycles or cycle length. Markov model estimates of stage length indicated a significant decrease in metestrus stage length (0.3 days) in the 168 mg/L group and a significant decrease in estrus stage length (0.4 days) in the 335 mg/L group relative to the control group. There were no corresponding changes to the other cycle stages (Appendix E). Given the minimal response and absence of an exposure concentration response, these findings are likely spurious.

Histopathology

This section describes the statistically significant or biologically noteworthy changes in the incidence of nonneoplastic lesions in the small intestine.

Small intestine: In male and female mice exposed to vanadyl sulfate, hyperplasia of the epithelium was observed in the ileum and jejunum of the small intestine; however, this lesion was not observed in the large intestine (Table 38). As with the other studies, the lesion was most prevalent in the ileum, followed by the jejunum. In males, epithelial hyperplasia was seen in the ileum in the ≥83.8 mg/L groups, with a positive trend and a significant pairwise comparison in the 335 mg/L group. In the jejunum, this lesion was seen in only one male in the 335 mg/L group. In females, epithelial hyperplasia of the ileum was observed in the ≥83.8 mg/L groups, with a positive trend and significant pairwise comparisons in the ≥168 mg/L groups. In the jejunum, epithelial hyperplasia was seen in the ≥168 mg/L groups, with a positive trend. In all applicable cases, the severity of the lesion increased with increasing exposure concentration. Incidences of epithelial hyperplasia were observed in all intestinal sites (combined) in males and females exposed to ≥83.8 mg/L, with a positive trend and significant pairwise comparisons in the 335 mg/L male group and the ≥168 mg/L female groups. This lesion was not observed in males and females exposed to ≤41.9 mg/L. Epithelial hyperplasia occurred in the ileum in all animals in the 335 mg/L groups, with the exception of one male. The histological appearance of this lesion was identical to that described in rats exposed to sodium metavanadate (see Sodium Metavanadate Rat Histopathology).

Table 38. Incidences of Epithelial Hyperplasia of the Small Intestine in Male and Female Mice in the Three-month Drinking Water Study of Vanadyl Sulfate

	0 mg/L	21.0 mg/L	41.9 mg/L	83.8 mg/L	168 mg/L	335 mg/L
Male						
n[a]	10	0	9	9	10[b]	10
Intestine, Small, Ileum						
Epithelium, hyperplasia[c]	0**	–[d]	0	2 (1.0)[e]	3 (1.3)	9** (1.9)
Intestine, Small, Jejunum						
Epithelium, hyperplasia	0	–	–	–	0	1 (2.0)
Intestine, Any Site						
Epithelium, hyperplasia	0**	–	0	2	3	9**
Female						
n	10	0	10	10[b]	10	10
Intestine, Small, Ileum						
Epithelium, hyperplasia	0**	–	0	1 (1.0)	5* (1.4)	10** (1.9)
Intestine, Small, Jejunum						
Epithelium, hyperplasia	0*	–	0	0	1 (1.0)	2 (2.0)
Intestine, Any Site	10					
Epithelium, hyperplasia	0**	–	0	1	5*	10**

Statistical significance for an exposed 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]Number of animals examined microscopically.
[b]For the small intestine, ileum data, n =9 for the male 168 mg/L group and the female 83.8 mg/L group.
[c]Number of animals with lesion. Statistical analyses performed by Cochran-Armitage (trend) and Fisher's exact (pairwise) one-sided tests.
[d]Data were not collected for an exposed group when animals exposed to higher concentrations exhibited no incidences of nonneoplastic lesions in that tissue.
[e]Average severity grade of observed lesion in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.

Genetic Toxicology

Data from all NTP genetic toxicity tests with sodium metavanadate and vanadyl sulfate are available in the NTP Chemical Effects in Biological Systems database: https://doi.org/10.22427/NTP-DATA-TOX-106.

The genetic toxicity of sodium metavanadate and vanadyl sulfate was evaluated in bacterial reverse mutation and in rat and mouse peripheral blood micronucleus assays.

Bacterial Mutation Studies

Neither sodium metavanadate nor vanadyl sulfate was mutagenic in *Salmonella typhimurium* strains TA98 and TA100 or in *Escherichia coli* strain WP2 *uvrA* (pKM101) in tests conducted with and without exogenous metabolic activation provided by phenobarbital/benzoflavone-induced male rat liver S9 and cofactors (Table D-1, Table D-2). Sodium metavanadate was tested at concentrations of 0.5–100 µg/plate in TA98 with or without S9 mix and in TA100 at concentrations of 0.5–100 µg/plate without S9 mix and 1.0–500 µg/plate with S9 mix. For TA98 and TA100, the top concentration was limited by cytotoxicity. In *E. coli*, sodium metavanadate was tested at concentrations of 40–6,000 µg/plate with or without S9 mix. Vanadyl sulfate was tested at concentrations of 0.4–100 µg/plate in TA98 and TA100 with or without S9 mix. For TA98, the top concentration was limited by cytotoxicity. In *E. coli*, vanadyl sulfate was tested at concentrations of 4.0–6,000 µg/plate with or without S9 mix.

In Vivo Peripheral Blood Micronucleus Test

At the end of the 3-month studies of sodium metavanadate and vanadyl sulfate, peripheral blood samples were obtained from male and female rats and mice and analyzed for the frequency of micronucleated immature erythrocytes (i.e., reticulocytes or polychromatic erythrocytes [PCEs]) and mature erythrocytes (i.e., normochromatic erythrocytes [NCEs]). In male and female rats, the reticulocyte population 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.

In the sodium metavanadate study, a significant trend test was observed for the frequency of micronucleated reticulocytes in male rats, meeting statistical criteria for an equivocal result (Table D-3). However, the frequencies of micronucleated reticulocytes for the control group and all groups exposed to sodium metavanadate were well within the laboratory historical vehicle control (mean ± 2 standard deviations, or 95% confidence interval); therefore, this result was judged to be negative. Significant increases in the percentage of reticulocytes were observed in male rats, indicating that exposure to sodium metavanadate had a stimulatory effect on erythropoiesis. No increases in the frequencies of micronucleated reticulocytes were observed in female rats after 3 months of exposure to sodium metavanadate via drinking water. No increases in the frequencies of micronucleated reticulocytes or micronucleated mature erythrocytes were observed in male or female mice exposed to sodium metavanadate in drinking water for 3 months (Table D-4). However, significant increases in the percentage of reticulocytes were observed for both male and female mice, indicating that exposure to sodium metavanadate had a stimulatory effect on erythropoiesis.

In the vanadyl sulfate study, male and female rats did not show an increase in micronucleated reticulocytes (Table D-5). No significant changes in the percentage of reticulocytes were observed for male or female rats, suggesting that vanadyl sulfate exposure did not affect erythropoiesis. No significant increases in the frequencies of micronucleated reticulocytes or micronucleated mature erythrocytes were observed in male or female mice administered vanadyl sulfate in drinking water for 3 months (Table D-6). However, significant increases in the percentage of reticulocytes were observed for male and female mice, indicating that exposure to vanadyl sulfate had a stimulatory effect on erythropoiesis.

Discussion

Vanadium occurs naturally in the earth's crust and is found in coal and petroleum crude oils, which can lead to background and anthropogenic contributions to oral human exposure.[8] The primary forms of vanadium identified in foodstuff have been characterized and reported to be vanadyl (V^{4+}) and vanadate (V^{5+}), likely because they are the more stable oxidation states.[10] The studies reported here were designed to address data needs for V^{4+} and V^{5+} compounds under comparable experimental designs and test settings. Vanadyl sulfate and sodium metavanadate were selected as representative test articles for the V^{4+} and V^{5+} forms, respectively. The studies describe 3-month drinking water evaluations of both test articles in Sprague Dawley (Hsd:Sprague Dawley® SD®) rats (including a perinatal phase) and B6C3F1/N mice.

Drinking water concentrations of the test articles ranged from 0 to 500 mg/L and from 0 to 335 mg/L for sodium metavanadate and vanadyl sulfate, respectively. There were notable reductions in water consumption at the highest concentration of both test articles for rats and mice, ranging from 10% to 28% (study average; postnatal day 28–112 [rats] and study day 0–91 [mice]) compared to the respective control groups. Table 39 lists the approximate daily dose of vanadium intake across studies as calculated using water consumption and percent vanadium by test article. Vanadium intake for a given compound across both species and sexes was similar. Furthermore, when comparing vanadium intake between the two test articles, intake was similar for the four lowest concentrations of sodium metavanadate and the four highest concentrations of vanadyl sulfate. For example, in male rats, vanadium intake at 31.3 mg/L sodium metavanadate (1.2 mg vanadium/kg/day) was similar to vanadium intake at 41.9 mg/L vanadyl sulfate (1.2 mg vanadium/kg/day).

Table 39. Daily Vanadium (mg/kg) Intake Based on Water Consumption

Test Compound (mg/L)	Vanadium (mg/L)[a]	Vanadium Intake (mg/kg/day)[b]		Vanadium Intake (mg/kg/day)[c]	
		Male Rats	Female Rats	Male Mice	Female Mice
Sodium Metavanadate					
0	0.0	0.0	0.0	0.0	0.0
31.3	13.1	1.2	1.3	1.4	1.3
62.5	26.1	2.3	2.7	2.8	2.8
125	52.1	4.5	5.3	5.5	5.6
250	104.3	8.0	8.9	11.1	10.8
500	208.5	15.1	15.8	19.4	19.0
Vanadyl Sulfate					
0	0.0	0.0	0.0	0.0	0.0
21.0	6.5	0.6	0.6	0.7	0.7
41.9	13.0	1.2	1.4	1.5	1.3
83.8	26.0	2.2	2.9	3.0	2.6
168	52.1	4.3	5.3	6.2	5.3
335	103.9	7.8	9.3	10.7	10.7

[a]Concentration of vanadium in drinking water calculated using vanadium composition per test article: 41.7% for sodium metavanadate and 31% for vanadyl sulfate.
[b]Calculated as vanadium concentration (mg/L) × water consumed (g/kg/day; postnatal day 28–112) and a solution density of 1 g/mL.
[c]Calculated as vanadium concentration (mg/L) × water consumed (g/kg/day; study day 0–91) and a solution density of 1 g/mL.

There was a consistent response across studies when evaluating the dose response of certain effects. An example is in Figure 6, which shows the percent incidence of hyperplasia in the ileum epithelium against vanadium intake (mg/kg/day) in all studies. Overall, the dose response for this endpoint follows a common trend, with the exception of the sodium metavanadate-male mice (SM-MM) study, which shows a shallower dose response and lower maximal effect.

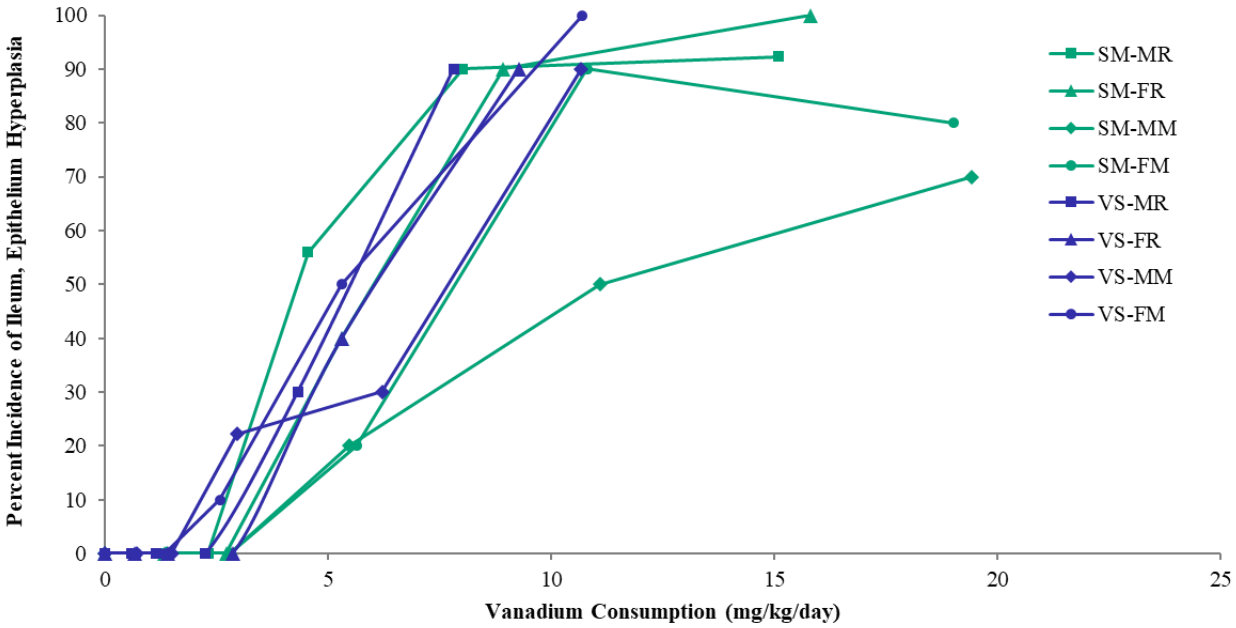


Figure 6. Percent Incidence of Epithelial Hyperplasia in the Ileum of Male and Female Rats and Mice Exposed to Sodium Metavanadate or Vanadyl Sulfate

SM = sodium metavanadate; VS = vanadyl sulfate; MR = male rats; FR = female rats; MM = male mice; FM = female mice.

The lowest-observed-effect levels (LOELs) for each study are shown in Table 40. The most sensitive endpoints tended to be related to erythrocyte microcytosis or epithelial hyperplasia of the gastrointestinal tract (largely the ileum). Erythrocyte microcytosis was observed in male mice exposed to the lowest concentration of sodium metavanadate; consequently, a no-observed-effect level was not obtained.

Table 40. Lowest-Observed-Effect Levels in Male and Female Rats and Mice in the Three-month Drinking Water Studies of Sodium Metavanadate and Vanadyl Sulfate

	Male Rats	Female Rats	Male Mice	Female Mice
Sodium Metavanadate				
LOEL				
Test article	125 mg/L	125 mg/L	31.3 mg/L	62.5 mg/L
Vanadium intake	4.5 mg/kg/day	5.3 mg/kg/day	1.4 mg/kg/day	2.8 mg/kg/day
Endpoint	Hematology Clinical chemistry Histopathology	Histopathology	Hematology	Hematology

	Male Rats	Female Rats	Male Mice	Female Mice
Effects[a]	↓ MCV ↑ Erythrocytes ↓ Cholesterol ↑ Ileum, epithelium hyperplasia ↑ Jejunum, epithelium hyperplasia	↑ Ileum, epithelium hyperplasia ↑ Jejunum, epithelium hyperplasia	↓ MCV	↓ MCV
Vanadyl Sulfate				
LOEL				
Test article	168 mg/L	168 mg/L	83.8 mg/L	83.8 mg/L
Vanadium intake	4.3 mg/kg/day	5.3 mg/kg/day	3.0 mg/kg/day	2.6 mg/kg/day
Endpoint	Histopathology	Histopathology	Histopathology	Hematology Histopathology
Effects	↑ Ileum, epithelium hyperplasia	↑ Ileum, epithelium hyperplasia	↑ Ileum, epithelium hyperplasia	↑ Erythrocytes ↑ Ileum, epithelium hyperplasia

LOEL = lowest-observed-effect level; MCV = mean cell volume.
[a]Selected LOELs for hematology are based on statistical significance. In some cases, histopathology LOELs reached statistical significance; however, significance was not required given there was no background incidence of the observed lesions. Effects considered secondary to dehydration (e.g., increased blood urea nitrogen) were not included.

In general, when considering vanadium intake, no consistent pattern was observed across all studies to indicate that total vanadium or test article oxidation state were major drivers for determining the dose at which effects occurred. In rats, the effect levels for histopathology between test articles aligned well with total consumed vanadium (Figure 7), indicating that total vanadium, rather than vanadium oxidation state, may be a driver for toxicity. For example, the calculated vanadium dose at which histopathological effects were seen ranged from 4.3 to 5.3 mg vanadium/kg/day for male and female rats exposed to either test article. However, there were some endpoints for which an effect was observed only in sodium metavanadate-exposed rats (e.g., hematology, clinical chemistry, and reduced dam and pup survival). This may be in part due to absorption, distribution, metabolism, and excretion differences between V^{4+} and V^{5+} compounds, wherein, at the end of these 3-month studies, up to threefold higher concentrations of vanadium in plasma and urine (measured in rats only) were observed in rats exposed to sodium metavanadate compared to rats exposed to vanadyl sulfate, when normalizing for vanadium intake per day (i.e., mg vanadium/day).[40] This pattern of higher concentrations of vanadium in plasma and urine from V^{5+} exposure compared to V^{4+} was also seen in shorter-term studies conducted as part of a National Toxicology Program (NTP) research program.[73] In mice, total vanadium intake did not seem to dictate the level at which an effect occurred. For both male and female mice, histopathology effects were observed at different calculated vanadium doses when comparing test articles (e.g., in female mice, the vanadyl sulfate LOEL for histopathology was 2.6 mg vanadium/kg/day versus the sodium metavanadate LOEL that was 5.6 mg vanadium/kg/day).

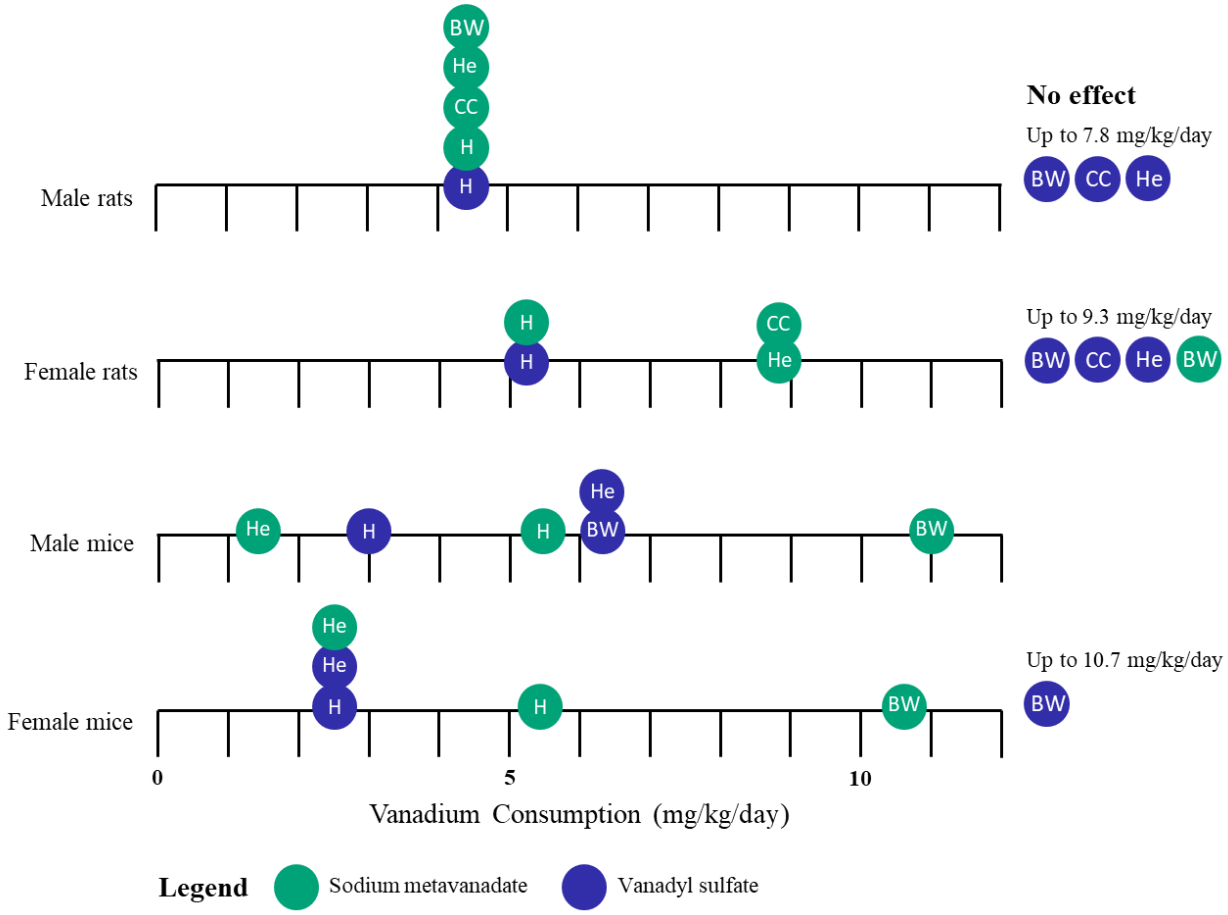


Figure 7. Effect Levels across Endpoints for Male and Female Rats and Mice in the Three-month Drinking Water Studies of Sodium Metavanadate or Vanadyl Sulfate

BW = body weight; CC = clinical chemistry; He = hematology; H = histopathology.

The intestinal epithelial hyperplasia observed across both test articles, sexes, and species is an unusual exposure-related lesion, especially in subchronic studies. Examples of NTP studies in which this lesion was observed include the 2-year study of nondecolorized whole-leaf extract of aloe vera in male and female rats (drinking water exposure),[74] the 3-month and 2-year studies of sodium dichromate dihydrate in male and female mice (drinking water exposure),[75; 76] and the 2-year study of D&C Yellow No. 11 in male rats (feed exposure).[77] In the D&C Yellow No. 11 studies, hyperplasia of the small intestinal epithelium was observed in F344/N rats in the 2-year core study, but not in the rats from the 12-month interim sacrifice; no neoplasms were observed in the small or large intestine in this study.[77] After 2 years of exposure via feed in male rats, D&C Yellow No. 11 induced a hyperplastic lesion in the small intestine that resembled that seen in the current study in which there were increased numbers of intestinal epithelial cells (not goblet cells) lining the small intestine. There were no neoplastic lesions in the small intestine, however. It is possible that, given more time, exposure to D&C Yellow No. 11 may have resulted in neoplasms of the small intestine. In the sodium dichromate dihydrate studies (mice only), exposure to the test article via drinking water for 2 years resulted in neoplasms of the small intestine (all segments combined). These neoplasms were accompanied by hyperplasia of the small intestinal epithelium,[75] which was also observed in the 3-month studies.[76] The

hyperplastic lesions in the small intestine were morphologically similar to that seen in the current study.

Three months of exposure to aloe vera extract in male and female F344/N rats and B6C3F1 mice and aloin in male F344/N rats mainly induced goblet cell hyperplasia in the large intestine.[78; 79] Goblet cell hyperplasia was not seen in the current study. In rats, 3 months of exposure to aloin or aloe vera extract resulted in mucosal hyperplasia of the large and small intestines, although it was much more prevalent in the large intestine.[78; 79] In studies on aloe vera nondecolorized whole-leaf extract, exposure for 3 months resulted in goblet cell hyperplasia in the large intestine of male and female F344/N rats and B6C3F1 mice.[74] In male and female rats, exposure for 2 years resulted in mucosa hyperplasia in the small and large intestines and neoplasms of the large intestine The mucosal hyperplasia in the large intestine induced by aloe vera appeared to be a thickened mucosal layer due to elongation of the crypts. No further description was provided. In the current study, the hyperplasia in the small and large intestines had a different appearance in that it was characterized by increased numbers of epithelial cells (not goblet cells). Furthermore, the main site of the hyperplasia in the current study was the ileum, with lower incidences in adjacent intestinal segments. The site of neoplasia in the aloe vera study at 2 years and the site with the highest incidence of hyperplasia after 13 weeks was the colon, which also differed from the current study.

The data from the sodium dichromate dihydrate and aloe vera studies suggest an association between hyperplasia and neoplasms, but this is not supported by the D&C Yellow No. 11 studies in which no neoplasms developed following 2 years of exposure. In all of these studies, however, intestinal epithelial hyperplasia was observed at the subchronic time point only in the sodium dichromate dihydrate study, exemplifying the rarity of seeing this lesion in a 3-month study. While the D&C Yellow No. 11 study shows that intestinal epithelial hyperplasia may not lead to neoplasia, the other two studies suggest such an association. Perhaps the most comparable studies are the sodium dichromate dihydrate studies because both sodium dichromate dihydrate and the vanadium compounds in the current study are metals, and they both resulted in hyperplastic intestinal lesions in mice. Therefore, given the similar appearance of these hyperplastic lesions to those seen in the sodium dichromate dihydrate and aloe vera nondecolorized whole-leaf extract studies, it is possible that longer term exposure to sodium metavanadate or vanadyl sulfate may result in the formation of intestinal neoplasms.

In the current drinking water studies, the hematology results indicated that exposure of mice to sodium metavanadate or vanadyl sulfate affected the circulating erythroid mass and resulted in a microcytic erythrocytosis effect. Interestingly, vanadium is one of ten transition metals in the "period 4" row of elements of the periodic table (in order: scandium [Sc], titanium [Ti], vanadium [V], chromium [Cr], manganese [Mn], iron [Fe], cobalt [Co], nickel [Ni], copper [Cu], and zinc [Zn]). Six of those transition elements, including vanadium, have been evaluated (in various forms and exposure paradigms) as part of NTP toxicity and carcinogenicity studies, including vanadium (as vanadium pentoxide),[1] chromium (as sodium dichromate dihydrate),[75; 76] cobalt (as cobalt sulfate heptahydrate and cobalt metal),[80-82] nickel (as nickel oxide and nickel sulfate hexahydrate),[83; 84] copper (as cupric sulfate),[85] and zinc (as dietary zinc).[86] In all of these NTP studies, there were some significant pairwise microcytic-type changes (either in rats or mice or in males or females). Significantly decreased mean cell volume was observed in the studies of vanadium, chromium, nickel, copper, and zinc, whereas erythrocytosis-type changes (i.e.,

significantly increased red blood cell count) were observed in the studies of vanadium, chromium, cobalt, nickel, and copper.

While the mechanism of these microcytic- and erythrocytosis-type changes are unknown, the idea that vanadium is in the same period 4 group of transition elements as iron should be considered. Iron is a key component of hemoglobin production. As an essential element of hemoglobin, an alteration in iron metabolism results in an iron deficiency (absolute or functional), which alters erythrocyte production and results in altered numbers and size (e.g., microcytic erythrocytes) of the circulating red blood cells. Iron is also an indispensable cofactor as an essential redox element that functions in many metabolic pathways. For example, the active hypoxia-inducible factor (HIF) hydroxylases, prolyl hydroxylase domains (PHDs), and factor inhibiting HIF (FIH) involved in the regulation of the HIF1α subunit of HIF require iron to be catalytically functional.[87] Cobalt, another "period 4" transition metal, is a well-known inducer of erythrocytosis in normoxic conditions related to the inhibition of PHDs and FIH, the stabilization of HIF1α, and the stimulation of erythropoietin by HIF1. Indeed, studies have shown that the active-site Fe^{2+} can be substituted by Co^{2+}, Cu^{2+}, Zn^{2+}, and Mn^{2+}. Therefore, the classic hypoxia-mimetic function (i.e., eythrocytosis through increased erythropoietin production) of cobalt appears to be explained by its direct inhibition of the HIF hydroxylases.[87] Interestingly, it has been demonstrated in vitro that the expression of HIF1α was induced by sodium orthovanadate.[88] Thus, vanadate administration may involve substitution of Fe and affect erythrocyte production through hemoglobin production and/or altered regulation of HIF.

Under the conditions of these studies, oral exposure to sodium metavanadate or vanadyl sulfate in drinking water resulted in hematological effects associated with erythrocyte microcytosis in rats (sodium metavanadate) and mice (sodium metavanadate and vanadyl sulfate), including erythrocytosis in male rats and in male and female mice. Epithelial hyperplasia of the ileum and other gastrointestinal sites was observed histologically, which was consistent for both test articles and across both sexes and species evaluated. In the sodium metavanadate studies, the LOELs were 125 mg/L in male and female rats, 31.3 mg/L in male mice, and 62.5 mg/L in female mice, based on changes in hematology (male rats and male and female mice) and epithelium hyperplasia in the ileum and jejunum (male and female rats). In the vanadyl sulfate studies, the LOELs were 168 mg/L in male and female rats and 83.8 mg/L in male and female mice, as indicated by epithelium hyperplasia in the ileum (male and female rats and mice) and hematology (female mice).

References

1. National Toxicology Program (NTP). Toxicology and carcinogenesis studies of vanadium pentoxide (CAS No. 1314-62-1) in F344/N rats and B6C3F1 mice (inhalation studies). Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health; 2002. NTP Technical Report No. 507. NIH Publication No. 03-4441. https://ntp.niehs.nih.gov/go/tr507abs

2. Roberts GK, Stout MD, Sayers B, Fallacara DM, Hejtmancik MR, Waidyanatha S, Hooth MJ. 14-day toxicity studies of tetravalent and pentavalent vanadium compounds in Harlan Sprague Dawley rats and B6C3F1/N mice via drinking water exposure. Toxicol Rep. 2016; 3:531-538. https://doi.org/10.1016/j.toxrep.2016.05.001

3. Roberts GK, Stout MD, Sayers B, Fallacara DM, Hejtmancik MR, Waidyanatha S, Hooth MJ. Clarification and lessons learned for reporting studies with hydrates. Citation: Roberts et al., 2016. Toxicology Reports 3: 531–538. Toxicol Rep. 2018; 5:207-208. https://doi.org/10.1016/j.toxrep.2017.12.023

4. National Center for Biotechnology Information (NCBI). PubChem Compound Summary for CID 34007, Vanadyl sulfate. Bethesda, MD: U.S. National Library of Medicine, National Center for Biotechnology Information; 2022. https://pubchem.ncbi.nlm.nih.gov/compound/34007

5. U.S. Environmental Protection Agency (USEPA). CompTox Chemicals Dashboard: Sodium metavanadate 13718-26-8 | DTXSID3044336. Washington, DC: U.S. Environmental Protection Agency; 2022. https://comptox.epa.gov/dashboard/chemical/details/DTXSID3044336

6. National Center for Biotechnology Information (NCBI). PubChem Compound Summary for CID 4148882, Sodium metavanadate. Bethesda, MD: U.S. National Library of Medicine, National Center for Biotechnology Information; 2022. https://pubchem.ncbi.nlm.nih.gov/compound/4148882

7. Rydzynski K, Pakulska D. Vanadium, niobium, and tantalum. In: Bingham E, Cohrssen B, editors. Patty's Toxicology. 6th ed. Hoboken, NJ: John Wiley & Sons; 2012. p. 511-564. https://doi.org/10.1002/0471435139.tox037.pub2

8. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for vanadium. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry; 2012. https://www.atsdr.cdc.gov/toxprofiles/tp58.pdf

9. Pennington JAT, Jones JW. Molybdenum, nickel, cobalt, vanadium, and strontium in total diets. J Am Diet Assoc. 1987; 87(12):1644-1650. https://doi.org/10.1016/S0002-8223(21)03381-2

10. Sepe A, Ciaralli L, Ciprotti M, Giordano R, Funari E, Costantini S. Determination of cadmium, chromium, lead and vanadium in six fish species from the Adriatic Sea. Food Addit Contam. 2003; 20(6):543-552. https://doi.org/10.1080/0265203031000069797

11. Office of Dietary Supplements (ODS). Dietary Supplement Label Database (DSLD). Bethesda, MD: National Institutes of Health, Office of Dietary Supplements; 2022. https://dsld.od.nih.gov/

12. Kothandan S, Sheela A. DNA interaction and cytotoxic studies on mono/di-oxo and peroxo-vanadium (V) complexes - A review. Mini Rev Med Chem. 2021; 21(14):1909-1924. http://doi.org/10.2174/1389557521666210308143522

13. Adachi A, Asai K, Koyama Y, Matsumoto Y, Okano T. Subacute vanadium toxicity in rats. J Health Sci. 2000; 46(6):503-508. https://doi.org/10.1248/jhs.46.503

14. Ścibior A, Zaporowska H, Ostrowski J. Selected haematological and biochemical parameters of blood in rats after subchronic administration of vanadium and/or magnesium in drinking water. Arch Environ Contam Toxicol. 2006; 51(2):287-295. https://doi.org/10.1007/s00244-005-0126-4

15. Domingo JL, Paternain JL, Llobet JM, Corbella J. Effects of vanadium on reproduction, gestation, parturition and lactation in rats upon oral administration. Life Sci. 1986; 39(9):819-824. https://doi.org/10.1016/0024-3205(86)90460-1

16. Elfant M, Keen CL. Sodium vanadate toxicity in adult and developing rats: Role of peroxidative damage. Biol Trace Elem Res. 1987; 14(3):193-208. https://doi.org/10.1007/BF02795686

17. Poggioli R, Arletti R, Bertolini A, Frigeri C, Benelli A. Behavioral and developmental outcomes of prenatal and postnatal vanadium exposure in the rat. Pharmacol Res. 2001; 43(4):341-347. https://doi.org/10.1006/phrs.2000.0788

18. Boscolo P, Carmignani M, Volpe AR, Felaco M, Del Rosso G, Porcelli G, Giuliano G. Renal toxicity and arterial hypertension in rats chronically exposed to vanadate. Occup Environ Med. 1994; 51(7):500-503. http://doi.org/10.1136/oem.51.7.500

19. Carmignani M, Boscolo P, Volpe AR, Togna G, Masciocco L, Preziosi P. Cardiovascular system and kidney as specific targets of chronic exposure to vanadate in the rat: Functional and morphological findings. In: Chambers PL, Chambers CM, Wiezorek WD, Golbs S, editors. Recent Developments in Toxicology: Trends, Methods and Problems. Berlin, Germany: Springer-Verlag; 1991. p. 124-127. https://doi.org/10.1007/978-3-642-74936-0_24

20. Carmignani M, Volpe AR, Porcelli G, Boscolo P, Preziosi P. Chronic exposure to vanadate as factor of arterial hypertension in the rat: Toxicodynamic mechanisms. In: Bolt HM, de Wolff FA, Henderson PT, editors. Medical Toxicology: Proceedings of the 1991 EUROTOX Congress Meeting Held in Masstricht, September 1–4, 1991. Berlin, Germany: Springer-Verlag; 1992. p. 117-120. https://doi.org/10.1007/978-3-642-77260-3_15

21. Akgün-Dar K, Bolkent S, Yanardag R, Tunalı S. Vanadyl sulfate protects against streptozotocin-induced morphological and biochemical changes in rat aorta. Cell Biochem Funct. 2007; 25(6):603-609. https://doi.org/10.1002/cbf.1354

22. Sanchez DJ, Colomina MT, Domingo JL. Effects of vanadium on activity and learning in rats. Physiol Behav. 1998; 63(3):345-350. https://doi.org/10.1016/S0031-9384(97)00433-2

23. Sanchez DJ, Colomina MT, Domingo JL, Corbella J. Prevention by sodium 4,5-dihydroxybenzene1,3-disulfonate (tiron) of vanadium-induced behavioral toxicity in rats. Biol Trace Elem Res. 1999; 69(3):249-259. https://doi.org/10.1007/BF02783877

24. U.S. Environmental Protection Agency (USEPA). Drinking water Contaminant Candidate List (CCL) and regulatory determination. Washington, DC: U.S. Environmental Protection Agency; 2021. https://www.epa.gov/ccl

25. U.S. Environmental Protection Agency (USEPA). Contaminant Candidate List 1 - CCL 1. Washington, DC: U.S. Environmental Protection Agency; 2022. https://www.epa.gov/ccl/contaminant-candidate-list-1-ccl-1

26. U.S. Environmental Protection Agency (USEPA). IRIS assessment plan for oral exposure to vanadium and compounds (scoping and problem formulation materials). Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development, Center for Public Health and Environmental Assessment, Integrated Risk Information System; 2020. EPA Report No. EPA/635/R-20/112. https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=P1010XZ0.txt

27. Treviño S, Díaz A, Sánchez-Lara E, Sanchez-Gaytan BL, Perez-Aguilar JM, González-Vergara E. Vanadium in biological action: Chemical, pharmacological aspects, and metabolic implications in diabetes mellitus. Biol Trace Elem Res. 2019; 188(1):68-98. https://doi.org/10.1007/s12011-018-1540-6

28. Adachi A, Ogawa K, Tsushi Y, Nagao N, Okano T. Balance, excretion and tissue distribution of vanadium in rats after short-term ingestion. J Health Sci. 2000; 46(1):59-62. https://doi.org/10.1248/jhs.46.59

29. Bogden JD, Higashino H, Lavenhar MA, Bauman JW Jr, Kemp FW, Aviv A. Balance and tissue distribution of vanadium after short-term ingestion of vanadate. J Nutr. 1982; 112(12):2279-2285. https://doi.org/10.1093/jn/112.12.2279

30. Parker RD, Sharma RP. Accumulation and depletion of vanadium in selected tissues of rats treated with vanadyl sulfate and sodium orthovanadate. J Environ Pathol Toxicol. 1978; 2(2):235-245.

31. Hamel FG, Duckworth WC. The relationship between insulin and vanadium metabolism in insulin target tissues. Mol Cell Biochem. 1995; 153(1-2):95-102. https://doi.org/10.1007/bf01075923

32. Ramanadham S, Heyliger C, Gresser MJ, Tracey AS, McNeill JH. The distribution and half-life for retention of vanadium in the organs of normal and diabetic rats orally fed vanadium(IV) and vanadium(V). Biol Trace Elem Res. 1991; 30(2):119-124. https://doi.org/10.1007/bf02990348

33. Azay J, Brès J, Krosniak M, Teissedre PL, Cabanis JC, Serrano JJ, Cros G. Vanadium pharmacokinetics and oral bioavailability upon single-dose administration of vanadyl sulfate to rats. Fundam Clin Pharmacol. 2001; 15(5):313-324. https://doi.org/10.1046/j.1472-8206.2001.00043.x

34. Paternain JL, Domingo JL, Gómez M, Ortega A, Corbella J. Developmental toxicity of vanadium in mice after oral administration. J Appl Toxicol. 1990; 10(3):181-186. https://doi.org/10.1002/jat.2550100307

35. Dimond EG, Caravaca J, Benchimol A. Vanadium, excretion, toxicity, lipid effect in man. Am J Clin Nutr. 1963; 12(1):49-53. https://doi.org/10.1093/ajcn/12.1.49

36. Willsky GR, Halvorsen K, Godzala ME 3rd, Chi LH, Most MJ, Kaszynski P, Crans DC, Goldfine AB, Kostyniak PJ. Coordination chemistry may explain pharmacokinetics and clinical response of vanadyl sulfate in type 2 diabetic patients. Metallomics. 2013; 5(11):1491-1502. https://doi.org/10.1039/c3mt00162h

37. Mutlu E, Cristy T, Graves SW, Hooth MJ, Waidyanatha S. Characterization of aqueous formulations of tetra- and pentavalent forms of vanadium in support of test article selection in toxicology studies. Environ Sci Pollut Res Int. 2017; 24(1):405-416. https://doi.org/10.1007/s11356-016-7803-x

38. Maronpot RR, Boorman GA. Interpretation of rodent hepatocellular proliferative alterations and hepatocellular tumors in chemical safety assessment. Toxicol Pathol. 1982; 10(2):71-78. https://doi.org/10.1177/019262338201000210

39. Boorman GA, Haseman JK, Waters MD, Hardisty JF, Sills RC. Quality review procedures necessary for rodent pathology databases and toxicogenomic studies: The National Toxicology Program experience. Toxicol Pathol. 2002; 30(1):88-92. https://doi.org/10.1080/01926230252824752

40. Waidyanatha S, Weber FX, Fallacara DM, Harrington JM, Levine K, Robinson VG, Sparrow BR, Stout MD, Fernando R, Hooth MJ, et al. Systemic exposure and urinary excretion of vanadium following perinatal subchronic exposure to vanadyl sulfate and sodium metavanadate via drinking water. Toxicol Lett. 2022; 360:53-61. https://doi.org/10.1016/j.toxlet.2022.03.004

41. Gart JJ, Chu KC, Tarone RE. Statistical issues in interpretation of chronic bioassay tests for carcinogenicity. J Natl Cancer Inst. 1979; 62(4):957-974. https://doi.org/10.1093/jnci/62.4.957

42. Armitage P. Statistical methods in medical research. Oxford, UK: Blackwell Scientific; 1971.

43. Dixon WJ, Massey FJ. Introduction to statistical analysis. 2nd ed. New York, NY: McGraw-Hill; 1957.

44. Tukey J. Easy summaries--numerical and graphical. In: Exploratory Data Analysis. Reading, MA: Addison-Wesley; 1977. p. 27-56.

45. Dunnett CW. A multiple comparison procedure for comparing several treatments with a control. J Am Stat Assoc. 1955; 50(272):1096-1121. http://dx.doi.org/10.1080/01621459.1955.10501294

46. Williams DA. A test for differences between treatment means when several dose levels are compared with a zero dose control. Biometrics. 1971; 27(1):103-117. http://dx.doi.org/10.2307/2528930

47. Williams DA. The comparison of several dose levels with a zero dose control. Biometrics. 1972; 28(2):519-531. http://dx.doi.org/10.2307/2556164

48. Hsu JC. The factor analytic approach to simultaneous inference in the general linear model. J Comput Graph Stat. 1992; 1(2):151-168. https://doi.org/10.1080/10618600.1992.10477011

49. Shirley E. A non-parametric equivalent of Williams' test for contrasting increasing dose levels of a treatment. Biometrics. 1977; 33(2):386-389. http://dx.doi.org/10.2307/2529789

50. Williams DA. A note on Shirley's nonparametric test for comparing several dose levels with a zero-dose control. Biometrics. 1986; 42(1):183-186. http://dx.doi.org/10.2307/2531254

51. Dunn OJ. Multiple comparisons using rank sums. Technometrics. 1964; 6(3):241-252. http://dx.doi.org/10.1080/00401706.1964.10490181

52. Jonckheere AR. A distribution-free k-sample test against ordered alternatives. Biometrika. 1954; 41(1-2):133-145. http://dx.doi.org/10.1093/biomet/41.1-2.133

53. Davison AC, Hinkley DV. Bootstrap methods and their application. Cambridge, UK: Cambridge University Press; 1997.

54. Datta S, Satten GA. Rank-sum tests for clustered data. J Am Stat Assoc. 2005; 100(471):908-915. https://doi.org/10.1198/016214504000001583

55. Hommel G. A stagewise rejective multiple test procedure based on a modified Bonferroni test. Biometrika. 1988; 75(2):383-386. https://doi.org/10.1093/biomet/75.2.383

56. Hothorn LA. Statistical evaluation of toxicological bioassays – a review. Toxicol Res (Camb). 2014; 3(6):418-432. https://doi.org/10.1039/c4tx00047a

57. Kalbfleisch JD, Lawless JF. The analysis of panel data under a Markov assumption. J Am Stat Assoc. 1985; 80(392):863-871. https://doi.org/10.1080/01621459.1985.10478195

58. Code of Federal Regulations (CFR). 21(Part 58).

59. Miller JA, Miller EC. Ultimate chemical carcinogens as reactive mutagenic electrophiles. In: Hiatt HH, Watson JD, Winsten JA, editors. Origins of Human Cancer. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory; 1977. p. 605-627.

60. Crawford BD. Perspectives on the somatic mutation model of carcinogenesis. In: Flamm WG, Lorentzen RJ, editors. Mechanisms and Toxicity of Chemical Carcinogens and Mutagens. Princeton, NJ: Princeton Scientific Publishing; 1985. p. 13-59.

61. Straus DS. Somatic mutation, cellular differentiation, and cancer causation. J Natl Cancer Inst. 1981; 67(2):233-241. https://doi.org/10.1093/jnci/67.2.233

62. Ashby J, Tennant RW. Definitive relationships among chemical structure, carcinogenicity and mutagenicity for 301 chemicals tested by the U.S. NTP. Mutat Res. 1991; 257(3):229-306. https://doi.org/10.1016/0165-1110(91)90003-e

63. Tennant RW, Margolin BH, Shelby MD, Zeiger E, Haseman JK, Spalding J, Caspary W, Resnick M, Stasiewicz S, Anderson B, et al. Prediction of chemical carcinogenicity in rodents

from in vitro genetic toxicity assays. Science. 1987; 236(4804):933-941. https://doi.org/10.1126/science.3554512

64. Zeiger E, Haseman JK, Shelby MD, Margolin BH, Tennant RW, Holden HE. Evaluation of four in vitro genetic toxicity tests for predicting rodent carcinogenicity: Confirmation of earlier results with 41 additional chemicals. Environ Mol Mutagen. 1990; 16 Suppl 18:1-14. https://doi.org/10.1002/em.2850160502

65. Heddle JA, Hite M, Kirkhart B, Mavournin K, MacGregor JT, Newell GW, Salamone MF. The induction of micronuclei as a measure of genotoxicity: A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat Res. 1983; 123(1):61-118. https://doi.org/10.1016/0165-1110(83)90047-7

66. Schmid W. The micronucleus test. Mutat Res. 1975; 31(1):9-15. https://doi.org/10.1016/0165-1161(75)90058-8

67. Shelby MD, Erexson GL, Hook GJ, Tice RR. Evaluation of a three-exposure mouse bone marrow micronucleus protocol: Results with 49 chemicals. Environ Mol Mutagen. 1993; 21(2):160-179. https://doi.org/10.1002/em.2850210210

68. Shelby MD, Witt KL. Comparison results from mouse bone marrow chromosome aberration and micronucleus tests. Environ Mol Mutagen. 1995; 25(4):302-313. https://doi.org/10.1002/em.2850250407

69. Witt KL, Knapton A, Wehr CM, Hook GJ, Mirsalis J, Shelby MD, MacGregor JT. Micronucleated erythrocyte frequency in peripheral blood of B6C3F(1) mice from short-term, prechronic, and chronic studies of the NTP carcinogenesis bioassay program. Environ Mol Mutagen. 2000; 36(3):163-194. https://doi.org/10.1002/1098-2280(2000)36:3<163::AID-EM1>3.0.CO;2-P

70. National Toxicology Program (NTP). TOX-106: Toxicity report tables & curves: Pathology tables, survival and growth curves from NTP short-term and genetic toxicology studies. Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program; 2023. https://doi.org/10.22427/NTP-DATA-TOX-106

71. National Toxicology Program (NTP). Modified one-generation study of 2-ethylhexyl p-methoxycinnamate (CASRN 5466-77-3) administered in feed to Sprague Dawley (Hsd:Sprague Dawley® SD®) rats with prenatal, reproductive performance, and subchronic assessments in F1 offspring. Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program; 2022. NTP Developmental and Reproductive Toxicity (DART) Technical Report No. 06. https://ntp.niehs.nih.gov/go/dart06abs

72. National Toxicology Program (NTP). Modified one-generation study of bisphenol AF (CASRN 1478-61-1) administered in feed to Sprague Dawley (Hsd:Sprague Dawley® SD®) rats with prenatal, reproductive performance, and subchronic assessments in F1 offspring. Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program; 2022. NTP Developmental and Reproductive Toxicity (DART) Technical Report No. 08. https://ntp.niehs.nih.gov/go/dart08abs

73. Harrington JM, Haines LG, Levine KE, Liyanapatirana C, Essader AS, Fernando RA, Robinson VG, Roberts GK, Stout MD, Hooth MJ, et al. Internal dose of vanadium in rats following repeated exposure to vanadyl sulfate and sodium orthovanadate via drinking water. Toxicol Appl Pharmacol. 2021; 412:115395. https://doi.org/10.1016/j.taap.2021.115395

74. National Toxicology Program (NTP). Toxicology and carcinogenesis studies of nondecolorized whole leaf extract of *Aloe barbadensis* Miller (aloe vera) in F344/N rats and B6C3F1 mice (drinking water studies). Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health; 2013. NTP Technical Report No. 577. NIH Publication No. 13-5910. https://ntp.niehs.nih.gov/go/tr577abs

75. National Toxicology Program (NTP). Toxicology and carcinogenesis studies of sodium dichromate dihydrate (CAS No. 7789-12-0) in F344/N rats and B6C3F1 mice (drinking water studies). Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health; 2008. NTP Technical Report No. 546. NIH Publication No. 08-5887. https://ntp.niehs.nih.gov/go/tr546abs

76. National Toxicology Program (NTP). Toxicity studies of sodium dichromate dihydrate (CAS No. 7789-12-0) administered in drinking water to male and female F344/N rats and B6C3F1 mice and male BALB/c and *am3*-C57BL/6 mice. Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health; 2007. NTP Toxicity Report No. 72. NIH Publication No. 07-5964. https://ntp.niehs.nih.gov/go/tox72abs

77. National Toxicology Program (NTP). Toxicology and carcinogenesis studies of D&C Yellow No. 11 (CAS No. 8003-22-3) in F344/N rats (feed studies). Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health; 1997. NTP Technical Report No. 463. NIH Publication No. 97-3379. https://ntp.niehs.nih.gov/go/tr463abs

78. Boudreau MD, Mellick PW, Olson GR, Felton RP, Thorn BT, Beland FA. Clear evidence of carcinogenic activity by a whole-leaf extract of Aloe barbadensis Miller (aloe vera) in F344/N rats. Toxicol Sci. 2013; 131(1):26-39. https://doi.org/10.1093/toxsci/kfs275

79. Boudreau MD, Olson GR, Tryndyak VP, Bryant MS, Felton RP, Beland FA. From the cover: Aloin, a component of the aloe vera plant leaf, induces pathological changes and modulates the composition of microbiota in the large intestines of F344/N male rats. Toxicol Sci. 2017; 158(2):302-318. https://doi.org/10.1093/toxsci/kfx105

80. National Toxicology Program (NTP). Toxicity studies of cobalt sulfate heptahydrate (CAS No. 10026-24-1) in F344/N rats and B6C3F1 mice (inhalation studies). Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health; 1991. NTP Toxicity Report No. 05. NIH Publication No. 91-3124. https://ntp.niehs.nih.gov/go/tox05abs

81. National Toxicology Program (NTP). Toxicology and carcinogenesis studies of cobalt sulfate heptahydrate (CAS No. 10026-24-1) in F344/N rats and B6C3F1 mice (inhalation studies). Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health; 1998. NTP Technical Report No. 471. NIH Publication No. 98-3961. https://ntp.niehs.nih.gov/go/tr471abs

82. National Toxicology Program (NTP). Toxicology studies of cobalt metal (CASRN 7440-48-4) in F344/N rats and B6C3F1/N mice and toxicology and carcinogenesis studies of cobalt metal in F344/NTac rats and B6C3F1/N mice (inhalation studies). Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program; 2014. NTP Technical Report No. 581. https://ntp.niehs.nih.gov/go/tr581abs

83. National Toxicology Program (NTP). Toxicology and carcinogenesis studies of nickel oxide (CAS No. 1313-99-1) in F344/N rats and B6C3F1 mice (inhalation studies). Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health; 1996. NTP Technical Report No. 451. NIH Publication No. 96-3367. https://ntp.niehs.nih.gov/go/tr451abs

84. National Toxicology Program (NTP). Toxicology and carcinogenesis studies of nickel sulfate hexahydrate (CAS No. 10101-97-0) in F344/N rats and B6C3F1 mice (inhalation studies). Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health; 1996. NTP Technical Report No. 454. NIH Publication No. 96-3370. https://ntp.niehs.nih.gov/go/tr454abs

85. National Toxicology Program (NTP). Toxicity studies of cupric sulfate (CAS No. 7758-99-8) administered in drinking water and feed to F344/N rats and B6C3F1 mice. Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health; 1993. NTP Toxicity Report No. 29. NIH Publication No. 93-3352. https://ntp.niehs.nih.gov/go/tox29abs

86. National Toxicology Program (NTP). Toxicology and carcinogenesis study of dietary zinc (CASRN 5263-02-5) in Sprague Dawley (Hsd:Sprague Dawley® SD®) rats (feed study). Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program; 2019. NTP Technical Report No. 592. https://ntp.niehs.nih.gov/go/tr592abs

87. Schofield CJ, Ratcliffe PJ. Oxygen sensing by HIF hydroxylases. Nat Rev Mol Cell Biol. 2004; 5(5):343-354. https://doi.org/10.1038/nrm1366

88. Gao N, Ding M, Zheng JZ, Zhang Z, Leonard SS, Liu KJ, Shi X, Jiang BH. Vanadate-induced expression of hypoxia-inducible factor 1 alpha and vascular endothelial growth factor through phosphatidylinositol 3-kinase/Akt pathway and reactive oxygen species. J Biol Chem. 2002; 277(35):31963-31971. https://doi.org/10.1074/jbc.M200082200

89. National Institute of Advanced Industrial Science and Technology (AIST). Spectral Database for Organic Compounds: SDBS-40459. Tokyo, Japan: National Institute of Advanced Industrial Science and Technology; 1999. https://sdbs.db.aist.go.jp/sdbs/cgi-bin/landingpage?sdbsno=40459

90. Sadtler Inorganics Library. YL No. 141. Hercules, CA: Bio-Rad Laboratories.

91. National Institute of Standards and Technology (NIST). NIST Standard Reference Database 69: NIST Chemistry WebBook: IR Spectrum: Sodium metavanadate. Gaithersburg, MD: U.S. Department of Commerce, National Institute of Standards and Technology; 2021. https://webbook.nist.gov/cgi/cbook.cgi?ID=B6000470&Units=SI&Mask=80#IR-Spec

92. Battelle. Powder Diffraction File-4+ release 2009 and the Inorganic Crystal Structure Database/NIST version 2009-2. Columbus, OH: Battelle; 2009.

93. Sadtler Inorganics Library. Reference spectrum for vanadyl sulfate trihydrate: SL No. 1424.

94. Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K. Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals. Environ Mol Mutagen. 1992; 19 Suppl 21:2-141. https://doi.org/10.1002/em.2850190603

95. Witt KL, Livanos E, Kissling GE, Torous DK, Caspary W, Tice RR, Recio L. Comparison of flow cytometry- and microscopy-based methods for measuring micronucleated reticulocyte frequencies in rodents treated with nongenotoxic and genotoxic chemicals. Mutat Res. 2008; 649(1-2):101-113. http://dx.doi.org/10.1016/j.mrgentox.2007.08.004

96. Dertinger SD, Camphausen K, MacGregor JT, Bishop ME, Torous DK, Avlasevich S, Cairns S, Tometsko CR, Menard C, Muanza T, et al. Three-color labeling method for flow cytometric measurement of cytogenetic damage in rodent and human blood. Environ Mol Mutagen. 2004; 44(5):427-435. https://doi.org/10.1002/em.20075

97. Kissling GE, Dertinger SD, Hayashi M, MacGregor JT. Sensitivity of the erythrocyte micronucleus assay: Dependence on number of cells scored and inter-animal variability. Mutat Res. 2007; 634(1-2):235-240. http://dx.doi.org/10.1016/j.mrgentox.2007.07.010

Appendix A. Chemical Characterization and Dose Formulation Studies

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A.1. Procurement and Characterization

A.1.1. Sodium Metavanadate

Sodium metavanadate was obtained from MP Biomedicals (Irvine, CA) in a single lot (8579K). Identity, purity, and stability analyses were conducted at the Battelle analytical chemistry laboratory (Columbus, OH). Reports on analyses performed in support of the sodium metavanadate studies are on file at the National Institute of Environmental Health Sciences (NIEHS).

Lot 8579K, a white-to-yellow powder, was identified as sodium metavanadate using infrared (IR) spectroscopy and X-ray diffraction (XRD). The IR spectrum was consistent with the library spectra (Figure A-1).[89-91] XRD analysis indicated that the sample was anhydrous sodium metavanadate because the indexed pattern matched the library pattern for metamunirite ($NaVO_3$) (Figure A-2).[92]

Moisture content was determined by weight loss on drying, Karl Fischer titration, and thermal gravimetric analysis (TGA). Moisture content by weight loss on drying was 0.2%. Under ambient conditions, the dried sample reabsorbed that same amount of water. Karl Fischer titration by Galbraith Laboratories, Inc. (Knoxville, TN) yielded a water content of 0.16%. TGA resulted in a measured water content of 0.25%.

Elemental analysis to aid in chemical identity confirmation and purity analysis was performed with scanning electron microscope (SEM) energy dispersive X-ray spectroscopy (EDS), proton-induced X-ray emission (PIXE) spectroscopy, and inductively coupled plasma (ICP) atomic emission spectroscopy (AES). The SEM-EDS spectrum was consistent with an inorganic compound consisting of vanadium (46.3%), sodium (28.5%), and oxygen (25.2%). No impurities were detected by SEM-EDS. The results for vanadium and sodium were higher compared to the theoretical values (41.78% and 18.86%, respectively). PIXE analysis by Elemental Analysis, Inc. (Lexington, KY) indicated the sample contains 34.9% vanadium, 20.5% sodium, and 44.6% oxygen. The percentage of vanadium was lower than the theoretical and the certificate of analysis (41.52%) values, and the percentage of sodium was higher than the theoretical value. No significant inorganic impurities were detected by PIXE. ICP-AES found the sample contained 41.3% vanadium and 18.9% sodium, which agreed with the theoretical and certificate of analysis values for sodium metavanadate.

Analysis using high-performance liquid chromatography (HPLC) with charged aerosol detection (CAD) (Table A-1, System A) showed a single peak with no reportable impurities. The volatile content was determined by gas chromatography (GC) with flame ionization detection (FID) and electron capture detection (ECD) (Table A-2, System G) using four halogenated and six nonhalogenated standards. No volatiles were detected with GC. The overall purity of lot 8579K was determined to be >99%.

Accelerated stability studies were conducted using HPLC/CAD (Table A-1, System A). Lot 8579K was stored at refrigerated (5°C), room (25°C), and elevated (60°C) temperatures for 2 weeks and then analyzed for purity relative to a frozen (−20°C) sample. Stability was confirmed for at least 2 weeks when stored sealed in amber glass at temperatures of ≤60°C. Three containers of lot 8579K were received and homogenized by mixing all contents in a 1-ft^3 Patterson-Kelley twin-shell blender for approximately 15 minutes. The bulk chemical was stored

at room temperature in amber plastic bottles. Reanalysis of the bulk chemical using HPLC/CAD (Table A-1, System B) was conducted before, during, and after the studies by the analytical chemistry laboratory. No significant differences were detected in the purities between the bulk test article and frozen reference standard during reanalysis.

A.1.2. Vanadyl Sulfate

Vanadyl sulfate was obtained from Noah Technologies Corporation (San Antonio, TX) in a single lot (0210324/1.1). The manufacturer's certificate of analysis for vanadyl sulfate provided the material as vanadyl sulfate hydrate with an unspecified number of water molecules ($VOSO_4 \cdot xH_2O$) but provided the CASRN for the anhydrous form because hydrated forms do not have a single CASRN. The chemical name vanadyl sulfate and the corresponding CASRN 27774-13-6 were used for the purpose of reporting the study. Identity, purity, and stability analyses were conducted at the Battelle analytical chemistry laboratory (Columbus, OH). Reports on analyses performed in support of the vanadyl sulfate studies are on file at NIEHS.

Lot 0210324/1.1, a blue, dusty powder, was identified as vanadyl sulfate using IR and XRD. The IR spectrum was consistent with the reference spectrum for vanadyl sulfate trihydrate from the Sadtler Inorganics Library (SL No. 1424) (Figure A-3).[93] The major absorption bands identified the presence of hydrated vanadyl sulfate in the test article. The XRD analysis indicated that the sample contained vanadyl sulfate in at least two hydrated crystalline phases: $(VO)(SO_4) \cdot 5H_2O$ and $(VO)(SO_4) \cdot 3H_2O$ (Figure A-4 and Figure A-5).[92] Anhydrous vanadyl sulfate was not detected by XRD.

Moisture content was determined by weight loss on drying, Karl Fischer titration, and TGA. Moisture content of the test article measured by weight loss on drying was 21.8%. Under ambient conditions, the dried sample reabsorbed 3.6% water. Karl Fischer titration by Galbraith Laboratories, Inc. (Knoxville, TN) yielded a water content of 22.8%. TGA resulted in a measured water content of 32.9%, which likely corresponded to both adsorbed water and water from the crystalline hydrates detected with XRD, whereas weight loss on drying and Karl Fischer titration measured only adsorbed water. Overall, lot 0120324/1.1 was determined to be 33% water and 67% vanadyl sulfate.

Elemental analysis to aid in chemical identity confirmation and purity analysis was performed with SEM-EDS, PIXE, and ICP-AES. SEM-EDS signals were present for only vanadium, sulfur, and oxygen in the sample, which is consistent with an identity of vanadyl sulfate without significant impurities. PIXE analysis by Elemental Analysis, Inc. (Lexington, KY) indicated the sample contained 19.5% vanadium and 14.2% sulfur, and no inorganic impurities were detected with concentrations >0.1%. ICP-AES analysis found the sample contained 22.1% vanadium and 14.3% sulfur, which agreed with the theoretical values for vanadium sulfate (20.94% and 13.18%, respectively), and consisted of 33% water.

Analysis using HPLC/CAD (Table A-1, System C) detected only vanadyl and sulfate ions. The volatile content was determined by GC/FID/ECD (Table A-2, System G) using four halogenated and six nonhalogenated standards. No volatiles were detected with GC. The overall purity of lot 0210324/1.1 was determined to be >99%.

Accelerated stability studies were conducted and analyzed using HPLC/CAD (Table A-1, System C). Lot 0210324/1.1 was stored in sealed amber glass vials at refrigerated (5°C), room

(25°C), and elevated (60°C) temperatures for 2 weeks and then analyzed for purity relative to a frozen (−20°C) sample. Stability was confirmed for at least 2 weeks when stored sealed in amber glass at temperatures of ≤60°C. Four containers of lot 0210324/1.1 were received and homogenized by mixing all contents in a 1-ft^3 Patterson-Kelley twin-shell blender for approximately 15 minutes. The bulk chemical was stored at room temperature in white plastic containers. Reanalysis of the bulk chemical using HPLC/CAD (Table A-1, System D) was performed before, during, and after the study by the analytical chemistry laboratory. No significant differences were detected in the purities between the bulk test article and frozen reference standard during reanalysis.

A.2. Preparation and Analysis of Dose Formulations

A.2.1. Sodium Metavanadate

The dose formulations for the perinatal (rats) and 3-month exposure (rats and mice) phases of the 3-month studies were prepared monthly at the Battelle study laboratory (West Jefferson, OH) by mixing sodium metavanadate with American Society for Testing and Materials (ASTM) Type I water, adjusted to pH 6 to 8 to give the required concentrations (Table A-3). Dose formulations of sodium metavanadate were prepared at six concentrations of 0, 31.3, 62.5, 125, 250, and 500 mg/L. The formulations were prepared five times for the rat study and three times for the mouse study. All dose formulations were stored at the study laboratory in plastic carboys at refrigerated temperatures (2°C–8°C) and were used within 42 days of preparation.

Stability studies were conducted on 10 mg/L and 30 mg/L sodium metavanadate dose formulations stored in polyethylene bottles protected from light. Formulation stability was measured after 42 days at refrigerated and room temperatures by the study laboratory using HPLC with ultraviolet (UV) detection (Table A-1, System E). Dose formulations were considered stable if the measured concentrations were within 10% of the day 0 concentration. Sodium metavanadate dose formulations were considered stable for up to 42 days when stored sealed at refrigerated or room temperatures and protected from light. A 7-day simulated dosing study was conducted to assess stability of 10 mg/L and 30 mg/L sodium metavanadate dose formulations by storing the formulations in a clear glass bottle with a sipper tube filled to near capacity and hung at room temperature. All sodium metavanadate formulations were considered stable for up to7 days under simulated animal room conditions.

Analysis of preadministration and postadministration (animal room) dose formulations were conducted monthly at the study laboratory according to the same procedure as the formulation stability study (Table A-1, System E). All preadministration dose formulations for sodium metavanadate (Table A-5, Table A-6) were within 10% of the target concentrations. All postadministration samples were within 10% of the target concentrations, except for two animal room samples for the rat study, which were 11.7% and 10.9% below the target concentrations.

A.2.2. Vanadyl Sulfate

The dose formulations for the perinatal (rats) and 3-month exposure (rats and mice) phases of the 3-month studies were prepared monthly at the Battelle study laboratory (West Jefferson, OH) by mixing vanadyl sulfate with ASTM Type I water, adjusted to pH 3.5 to give the required concentrations (Table A-3). Dose formulations of vanadyl sulfate were prepared at six concentrations of 0, 21.0, 41.9, 83.8, 168, and 335 mg/L (after being corrected for 33% water

content). The formulations were prepared five times for the rat study and three times for the mouse study. All dose formulations were stored at the study laboratory in plastic carboys at refrigerated temperatures (2°C–8°C) and were used within 42 days of preparation.

Stability studies were conducted on 10, 30, 50, and 2,000 mg/L vanadyl sulfate dose formulations stored in polyethylene bottles protected from light. Formulation stability was measured after 42 days at refrigerated and room temperatures by the study laboratory using HPLC/UV (Table A-1, Systems E and F). Dose formulations were considered stable if the measured concentrations were within 10% of the day 0 concentration. A small peak corresponding to the retention time of vanadate (V^{5+}) was observed in the 50 and 2,000 mg/L vanadyl sulfate formulations; the absolute amount of conversion was similar regardless of the concentration and did not increase with time.[37] Vanadyl sulfate dose formulations were considered stable for up to 42 days when stored sealed at refrigerated or room temperatures and protected from light. A 7-day simulated dosing study was conducted to assess stability of 10 mg/L and 30 mg/L vanadyl sulfate dose formulations by storing the formulations in a clear glass bottle with a sipper tube filled to near capacity and hung at room temperature. The 10 mg/L and 30 mg/L vanadyl sulfate formulations decreased in concentration by up to 20.7% and 11%, respectively, compared to day 0 concentrations. The appearance of a second chromatographic peak suggests that some oxidation of vanadyl (V^{4+}) to vanadate (V^{5+}) occurred and was the cause of the reduced test article concentration.

Analysis of preadministration and postadministration (animal room) dose formulations were conducted monthly at the study laboratory according to the same procedure as the formulation stability study (Table A-1, System E). All preadministration dose formulations of vanadyl sulfate (Table A-7, Table A-8) were within 10% of the target concentrations. All postadministration samples were within 10% of target concentrations, except for one animal room sample for the rat study, which was 13.7% below the target concentration.

Table A-1. Liquid Chromatography Systems Used in the Three-month Drinking Water Studies of Sodium Metavanadate and Vanadyl Sulfate

Chromatography	Detection System	Column	Mobile Phase
System A			
High-performance liquid chromatography[a]	Charged aerosol detector	Phenomenex Luna HILIC (150 mm × 4.6 mm, 5 µm particle size)	A: 77% acetonitrile with 0.1% formic acid and 50 mM ammonium formate B: ASTM Type I water with 0.1% formic acid and 50 mM ammonium formate Gradient program: A:B 95:5 to 75:25 in 3 minutes; hold at 75:25 for 4 minutes; 75:25 to 95:5 in 3 minutes; hold at 95:5 for 5 minutes, 1.0 mL/min flow rate

Chromatography	Detection System	Column	Mobile Phase
System B			
High-performance liquid chromatography[b]	Charged aerosol detector	Phenomenex Luna HILIC (150 mm × 4.6 mm, 5 µm particle size)	A: Acetonitrile:ASTM Type I water with 0.1% formic acid and 50 mM ammonium formate (77:23) B: ASTM Type I water with 0.1% formic acid and 50 mM ammonium formate Gradient program: A:B 95:5 to 75:25 in 3 minutes; hold at 75:25 for 4 minutes; 75:25 to 95:5 in 3 minutes; hold at 95:5 for 5 minutes, 1 mL/min flow rate
System C			
High-performance liquid chromatography[a]	Charged aerosol detector	Phenomenex Luna HILIC (150 mm × 4.6 mm, 5 µm particle size)	A: Acetonitrile B: Water, 3% formic acid, 50 mM ammonium formate, Gradient program: A:B Isocratic 75:22 for 30 minutes, 0.5 mL/min flow rate
System D			
High-performance liquid chromatography[b]	Charged aerosol detector	Phenomenex Luna HILIC (150 mm × 4.6 mm, 5 µm particle size)	A: Acetonitrile B: Water C: Formic acid and 50 mM ammonium formate Gradient program: A:B:C Isocratic 75:22:3 for 30 minutes, 0.5 mL/min flow rate
System E			
High-performance liquid chromatography[c]	Ultraviolet (282 nm)	Metrohm Metrosep A Sup 5 (50 mm × 4.0 mm, 5 µm particle size)	13.0–13.1 mM sodium bicarbonate, 4 mM sodium carbonate, 20 mM EDTA in ASTM Type I water, pH 6.6 Isocratic for 10 minutes, 1 mL/min flow rate
System F			
High-performance liquid chromatography[b]	Ultraviolet (282 nm)	Metrohm Metrosep A Sup 5 (50 mm × 4.0 mm, 5 µm particle size)	12 mM sodium bicarbonate, 4 mM sodium carbonate, 20 mM EDTA in ASTM Type I water, pH 6.6 Isocratic for 10 minutes, 1 mL/min flow rate

ASTM = American Society for Testing and Materials; EDTA = ethylenediaminetetraacetic acid.
[a]The liquid chromatographs, Waters Alliance Model 2695, were manufactured by Waters (Milford, MA).
[b]The liquid chromatographs, Agilent 1100, were manufactured by Agilent (Santa Clara, CA).
[c]The liquid chromatographs, Agilent 1260, were manufactured by Agilent (Santa Clara, CA).

Table A-2. Gas Chromatography System Used in the Three-month Drinking Water Studies of Sodium Metavanadate and Vanadyl Sulfate

Detection System	Column	Carrier Gas	Oven Temperature Program
System G			
Flame ionization (260°C) and electron capture detection (260°C)[a]	Restek Rtx-624 (30 m × 0.53 mm ID, 3 μm film thickness)	Helium at 5 mL/min	35°C for 14 minutes, then 15°C/min to 40°C, held for 3 minutes, then 15°C/min to 240°C, held for 2 minutes

ID = internal diameter.
[a]The gas chromatographs, Agilent 6890, were manufactured by Agilent (Santa Clara, CA).

Table A-3. Preparation and Storage of Dose Formulations Administered to Male and Female Rats in the Perinatal and Three-month Drinking Water Studies of Sodium Metavanadate and Vanadyl Sulfate

Sodium Metavanadate	Vanadyl Sulfate
Preparation	
The appropriate amount of test article was weighed and transferred into individual Nalgene® containers prefilled with the appropriate volume of vehicle. The weighing container was rinsed at least three times with the vehicle from the mixing container, and the rinse was added to the mixing container. The mixing container contents were blended with an overhead stirrer for ~15 minutes. Approximately 200 mL of formulation was dispensed through the spigot and poured back into the mixing container to ensure that the spigot was clear of any undissolved test article. After mixing, the pH of each formulation batch was measured and adjusted with hydrochloric acid or sodium hydroxide, as necessary. Formulations were prepared monthly throughout the studies.	
Vehicle	
ASTM Type I water, adjusted to within pH 6 to 8 with 37% hydrochloric acid and/or 10N sodium hydroxide while stirring with a drum stirrer.	ASTM Type I water, adjusted to pH 3.5 ± 0.1 using 37% hydrochloric acid and/or 10N sodium hydroxide.
Chemical Lot Number	
8579K (MP Biomedicals, Irvine, CA)	0210324/1.1 (Noah Technologies Corporation, San Antonio, TX)
Maximum Storage Time	
42 days	
Storage Conditions	
Plastic carboys stored refrigerated at 2°C–8°C.	
Analytical Laboratory	
Battelle (Columbus, OH)	
Study Laboratory	
Battelle (West Jefferson, OH)	

ASTM = American Society for Testing and Materials.

Table A-4. Preparation and Storage of Dose Formulations Administered to Male and Female Mice in the Three-month Drinking Water Studies of Sodium Metavanadate and Vanadyl Sulfate

Sodium Metavanadate	Vanadyl Sulfate
Preparation	
The appropriate amount of test article was weighed and transferred into individual Nalgene carboys, each equipped with a spigot, prefilled with 90% of the final volume of vehicle. The weighing container was rinsed at least three times with the vehicle from the mixing container, and the rinse was added to the mixing container. The mixing container contents were blended with an overhead stirrer for ~15 minutes. After mixing, ~200 mL of formulation was dispensed through the spigot and poured back into the mixing container to ensure that the spigot was clear of any undissolved test article. The formulations were diluted to final volume with vehicle and stirred for ~2 additional minutes. After additional mixing, the pH of each formulation batch was measured and adjusted with hydrochloric acid or sodium hydroxide, as necessary. Formulations were prepared monthly throughout the studies.	
Vehicle	
ASTM Type I water, adjusted to within pH 6 to 8 with 37% hydrochloric acid and/or 10N sodium hydroxide while stirring with a drum stirrer.	ASTM Type I water, adjusted to pH 3.5 ± 0.1 using 37% hydrochloric acid and/or 10N sodium hydroxide.
Chemical Lot Number	
8579K (MP Biomedicals, Irvine, CA)	0210324/1.1 (Noah Technologies Corporation, San Antonio, TX)
Maximum Storage Time	
42 days	
Storage Conditions	
Plastic carboys stored refrigerated at 2°C–8°C.	
Analytical Laboratory	
Battelle (Columbus, OH)	
Study Laboratory	
Battelle (West Jefferson, OH)	

ASTM = American Society for Testing and Materials.

Table A-5. Results of Analyses of Dose Formulations Administered to Male and Female Rats in the Perinatal and Three-month Drinking Water Study of Sodium Metavanadate

Date Prepared	Date Analyzed[a]	Target Concentration (mg/L)	Determined Concentration (mg/L)[b]	Difference from Target (%)
January 28, 2016	January 29, 2016	0	BLOQ	NA
		31.3	30.9 ± 0.2	−1.3
		62.5	60.2 ± 0.5	−3.7
		125	122 ± 1	−2.7
		250	239 ± 2	−4.4
		500	490 ± 5	−2.0
February 26, 2016	March 1, 2016	0	BLOQ	NA
		31.3	30.2 ± 0.2	−3.6
		62.5	58.7 ± 0.2	−6.1
		125	121 ± 1	−3.2
		250	240 ± 1	−4.1
		500	487 ± 2	−2.6
March 28, 2016	March 28, 2016	0	BLOQ	NA
		31.3	30.7 ± 0.2	−2.0
		62.5	58.2 ± 0.2	−6.9
		125	121 ± 0	−3.2
		250	238 ± 1	−4.7
		500	478 ± 3	−4.5
April 22, 2016	April 25, 2016	0	BLOQ	NA
		31.3	30.0 ± 0.1	−4.2
		62.5	61.5 ± 0.0	−1.6
		125	124 ± 0	−0.8
		250	242 ± 1	−3.1
		500	501 ± 3	0.3
May 19, 2016	May 20, 2016	0	BLOQ	NA
		31.3	29.2 ± 0.1	−6.7
		62.5	60.4 ± 0.6	−3.4
		125	119 ± 1	−4.5
		250	239 ± 1	−4.4
		500	477 ± 3	−4.7
Animal Room Samples				
January 28, 2016 (Drinking Water Bottle)	March 16, 2016	0	BLOQ	NA
		31.3	29.6 ± 0.2	−5.5

Date Prepared	Date Analyzed[a]	Target Concentration (mg/L)	Determined Concentration (mg/L)[b]	Difference from Target (%)
		62.5	55.2 ± 0.2	−11.7
		125	111 ± 2	−10.9
		250	224 ± 1	−10.4
		500	478 ± 0	−4.4
January 28, 2016 (Carboy)	March 16, 2016	0	BLOQ	NA
		31.3	30.8 ± 0.1	−1.7
		62.5	60.3 ± 0.3	−3.6
		125	119 ± 1	−4.5
		250	235 ± 1	−6.0
		500	485 ± 0	−3.0
February 26, 2016 (Drinking Water Bottle)	April 12, 2016	0	BLOQ	NA
		31.3	30.6 ± 0.2	−2.1
		62.5	59.0 ± 0.6	−5.5
		125	119 ± 1	−5.1
		250	230 ± 1	−8.0
		500	483 ± 3	−3.3
February 26, 2016 (Carboy)	April 12, 2016	0	BLOQ	NA
		31.3	30.4 ± 0.4	−2.9
		62.5	58.9 ± 0.1	−5.8
		125	121 ± 1	−2.9
		250	237 ± 2	−5.2
		500	489 ± 4	−2.3
March 28, 2016 (Drinking Water Bottle)	May 10, 2016	0	BLOQ	NA
		31.3	31.3 ± 0.1	0.1
		62.5	60.9 ± 0.1	−2.6
		125	124 ± 2	−0.5
		250	244 ± 1	−2.5
		500	483 ± 4	−3.5
March 28, 2016 (Carboy)	May 10, 2016	0	BLOQ	NA
		31.3	31.4 ± 0.1	0.2
		62.5	60.7 ± 0.4	−2.9
		125	125 ± 2	−0.3
		250	236 ± 1	−5.7
		500	484 ± 4	−3.1

Date Prepared	Date Analyzed[a]	Target Concentration (mg/L)	Determined Concentration (mg/L)[b]	Difference from Target (%)
April 22, 2016 (Drinking Water Bottle)	June 3, 2016	0	BLOQ	NA
		31.3	31.1 ± 0.1	−0.5
		62.5	62.4 ± 0.4	−0.2
		125	123 ± 1	−1.6
		250	243 ± 3	−2.7
		500	509 ± 4	1.7
April 22, 2016 (Carboy)	June 3, 2016	0	BLOQ	NA
		31.3	30.8 ± 0.1	−1.7
		62.5	62.5 ± 0.5	0.0
		125	127 ± 1	1.9
		250	242 ± 2	−3.2
		500	518 ± 16	3.5
May 19, 2016 (Drinking Water Bottle)	July 1, 2016	0	BLOQ	NA
		31.3	32.4 ± 0.2	3.6
		62.5	57.9 ± 0.1	−7.3
		125	122 ± 0	−2.4
		250	256 ± 3	2.5
		500	500 ± 5	0.1
May 19, 2016 (Carboy)	July 1, 2016	0	BLOQ	NA
		31.3	31.4 ± 0.7	0.4
		62.5	61.7 ± 0.7	−1.3
		125	126 ± 6	0.5
		250	240 ± 4	−4.0
		500	491 ± 3	−1.7

BLOQ = below the limit of quantification; NA = not applicable.
[a]Date first chromatograms were acquired.
[b]Data are presented as mean ± standard deviation of triplicate analysis.

Table A-6. Results of Analyses of Dose Formulations Administered to Male and Female Mice in the Three-month Drinking Water Study of Sodium Metavanadate

Date Prepared	Date Analyzed[a]	Target Concentration (mg/L)	Determined Concentration (mg/L)[b]	Difference from Target (%)
January 13, 2016	January 14, 2016	0	BLOQ	NA
		31.3	31.0 ± 0.3	−1.0
		62.5	62.1 ± 0.2	−0.6
		125	124 ± 1	−0.5
		250	236 ± 2	−5.6
		500	481 ± 4	−3.8
February 11, 2016	February 12, 2016	0	BLOQ	NA
		31.3	29.7 ± 0.6	−5.2
		62.5	60.5 ± 1.6	−3.1
		125	125 ± 1	0.3
		250	249 ± 1	−0.5
		500	498 ± 3	−0.5
March 15, 2016	March 15, 2016	0	BLOQ	NA
		31.3	31.2 ± 0.1	−0.3
		62.5	62.7 ± 0.4	0.3
		125	124 ± 1	−0.8
		250	248 ± 1	−0.7
		500	498 ± 3	−0.5
Animal Room Samples				
January 13, 2016 (Drinking Water Bottle)	February 24, 2016	0	BLOQ	NA
		31.3	31.8 ± 0.2	1.5
		62.5	62.9 ± 0.1	0.6
		125	125 ± 1	−0.3
		250	251 ± 2	0.4
		500	506 ± 4	1.2
January 13, 2016 (Carboy)	February 24, 2016	0	BLOQ	NA
		31.3	31.4 ± 0.3	0.4
		62.5	62.1 ± 0.7	−0.6
		125	122 ± 1	−2.1
		250	248 ± 2	−0.8
		500	498 ± 3	−0.5
February 11, 2016 (Drinking Water Bottle)	March 23, 2016	0	BLOQ	NA
		31.3	31.4 ± 0.2	0.2

Date Prepared	Date Analyzed[a]	Target Concentration (mg/L)	Determined Concentration (mg/L)[b]	Difference from Target (%)
		62.5	63.4 ± 0.5	1.5
		125	124 ± 1	−1.1
		250	245 ± 6	−2.1
		500	499 ± 1	−0.1
February 11, 2016 (Carboy)	March 23, 2016	0	BLOQ	NA
		31.3	31.2 ± 0.2	−0.3
		62.5	61.7 ± 0.2	−1.3
		125	124 ± 1	−0.5
		250	248 ± 2	−0.8
		500	501 ± 3	0.3
March 15, 2016 (Drinking Water Bottle)	April 27, 2016	0	BLOQ	NA
		31.3	31.9 ± 0.3	1.9
		62.5	63.7 ± 0.2	1.9
		125	123 ± 1	−1.3
		250	244 ± 1	−2.4
		500	480 ± 12	−4.1
March 15, 2016 (Carboy)	April 27, 2016	0	BLOQ	NA
		31.3	31.4 ± 0.2	0.4
		62.5	61.8 ± 0.3	−1.2
		125	123 ± 1	−1.9
		250	245 ± 4	−1.9
		500	479 ± 1	−4.3

BLOQ = below the limit of quantification; NA = not applicable.
[a]Date first chromatograms were acquired.
[b]Data are presented as mean ± standard deviation of triplicate analysis.

Table A-7. Results of Analyses of Dose Formulations Administered to Male and Female Rats in the Perinatal and Three-month Drinking Water Study of Vanadyl Sulfate

Date Prepared	Date Analyzed[a]	Target Concentration (mg/L)	Determined Concentration (mg/L)[b]	Difference from Target (%)
April 15, 2016	April 18, 2016	0	BLOQ	NA
		21.0	20.1 ± 0.2	−4.4
		41.9	39.5 ± 0.7	−5.6
		83.8	82.8 ± 1.3	−1.2
		168	159 ± 4	−5.2
		335	319 ± 5	−4.7
May 10, 2016	May 12, 2016	0	BLOQ	NA
		21.0	21.2 ± 0.3	0.8
		41.9	41.7 ± 0.6	−0.6
		83.8	83.8 ± 1.9	0.0
		168	170 ± 2	1.4
		335	339 ± 6	1.1
June 13, 2016	June 15, 2016	0	BLOQ	NA
		21.0	20.7 ± 0.0	−1.4
		41.9	39.2 ± 0.2	−6.4
		83.8	78.7 ± 0.6	−6.1
		168	158 ± 1	−5.8
		335	321 ± 2	−4.2
July 11, 2016	July 19, 2016	0	BLOQ	NA
		21.0	21.7 ± 0.9	3.2
		41.9	40.8 ± 0.3	−2.5
		83.8	82.5 ± 0.5	−1.6
		168	168 ± 4	0.4
		335	344 ± 1	2.8
August 3, 2016	August 5, 2016	0	BLOQ	NA
		21.0	20.1 ± 0.2	−4.4
		41.9	40.7 ± 0.2	−2.8
		83.8	82.7 ± 0.3	−1.4
		168	164 ± 1	−2.2
		335	329 ± 1	−1.7
Animal Room Samples				
April 15, 2016 (Drinking Water Bottle)	May 25, 2016	0	BLOQ	NA
		21.0	20.7 ± 0.1	−1.6

Date Prepared	Date Analyzed[a]	Target Concentration (mg/L)	Determined Concentration (mg/L)[b]	Difference from Target (%)
		41.9	40.6 ± 0.5	−3.1
		83.8	83.2 ± 0.6	−0.8
		168	165 ± 1	−1.6
		335	334 ± 1	−0.4
April 15, 2016 (Carboy)	May 25, 2016	0	BLOQ	NA
		21.0	20.8 ± 0.1	−1.0
		41.9	43.0 ± 2.0	2.7
		83.8	85.5 ± 2.6	2.0
		168	169 ± 1	0.4
		335	335 ± 6	0.1
May 10, 2016 (Drinking Water Bottle)	June 23, 2016	0	BLOQ	NA
		21.0	21.1 ± 0.1	0.3
		41.9	42.3 ± 0.3	0.9
		83.8	84.0 ± 0.5	0.2
		168	168 ± 0	0.0
		335	339 ± 4	1.3
May 10, 2016 (Carboy)	June 23, 2016	0	BLOQ	NA
		21.0	21.3 ± 0.1	1.3
		41.9	42.9 ± 0.1	2.3
		83.8	79.3 ± 6.8	−5.3
		168	174 ± 0	3.6
		335	344 ± 4	2.6
June 13, 2016 (Drinking Water Bottle)	July 26, 2016	0	BLOQ	NA
		21.0	20.4 ± 0.3	−2.8
		41.9	37.9 ± 0.1	−9.5
		83.8	76.5 ± 0.5	−8.7
		168	145 ± 1	−13.7
		335	317 ± 3	−5.5
June 13, 2016 (Carboy)	July 26, 2016	0	BLOQ	NA
		21.0	20.5 ± 0.3	−2.5
		41.9	38.4 ± 0.1	−8.3
		83.8	76.2 ± 0.3	−9.1
		168	154 ± 2	−8.1
		335	314 ± 6	−6.4

Date Prepared	Date Analyzed[a]	Target Concentration (mg/L)	Determined Concentration (mg/L)[b]	Difference from Target (%)
July 11, 2016 (Drinking Water Bottle)	August 23, 2016	0	BLOQ	NA
		21.0	21.4 ± 0.1	1.9
		41.9	41.2 ± 0.2	−1.6
		83.8	81.5 ± 0.9	−2.7
		168	159 ± 1	−5.2
		335	318 ± 0	−5.1
July 11, 2016 (Carboy)	August 23, 2016	0	BLOQ	NA
		21.0	21.3 ± 0.1	1.6
		41.9	39.9 ± 0.1	−4.8
		83.8	82.0 ± 0.5	−2.1
		168	168 ± 3	0.2
		335	340 ± 5	1.5
August 3, 2016 (Drinking Water Bottle)	September 16, 2016	0	BLOQ	NA
		21.0	19.6 ± 0.1	−6.7
		41.9	39.7 ± 0.5	−5.2
		83.8	79.7 ± 1.0	−4.9
		168	160 ± 2	−5.0
		335	321 ± 2	−4.2
August 3, 2016 (Carboy)	September 16, 2016	0	BLOQ	NA
		21.0	19.7 ± 0.2	−6.0
		41.9	40.4 ± 0.1	−3.5
		83.8	81.2 ± 0.8	−3.1
		168	161 ± 3	−4.2
		335	322 ± 9	−3.9

BLOQ = below the limit of quantification; NA = not applicable.
[a]Date first chromatograms were acquired.
[b]Data are presented as mean ± standard deviation of triplicate analysis.

Table A-8. Results of Analyses of Dose Formulations Administered to Male and Female Mice in the Three-month Drinking Water Study of Vanadyl Sulfate

Date Prepared	Date Analyzed[a]	Target Concentration (mg/L)	Determined Concentration (mg/L)[b]	Difference from Target (%)
April 4, 2016	April 5, 2016	0	BLOQ	NA
		21.0	21.2 ± 0.1	1.1
		41.9	41.5 ± 0.8	−0.9
		83.8	83.0 ± 2.2	−0.9
		168	168 ± 3	0.2
		335	323 ± 12	−3.5
April 28, 2016	April 28, 2016	0	BLOQ	NA
		21.0	21.1 ± 0.4	0.6
		41.9	42.4 ± 0.8	1.2
		83.8	85.5 ± 0.9	2.0
		168	172 ± 5	2.2
		335	344 ± 1	2.8
May 31, 2016	June 1, 2016	0	BLOQ	NA
		21.0	21.1 ± 0.3	0.6
		41.9	42.4 ± 0.4	1.1
		83.8	84.3 ± 1.2	0.6
		168	162 ± 1	−3.4
		335	334 ± 6	−0.2
Animal Room Samples				
April 4, 2016 (Drinking Water Bottle)	May 11, 2016	0	BLOQ	NA
		21.0	20.3 ± 0.3	−3.3
		41.9	39.9 ± 0.6	−4.8
		83.8	80.7 ± 0.8	−3.7
		168	163 ± 4	−3.2
		335	312 ± 4	−6.9
April 4, 2016 (Carboy)	May 11, 2016	0	BLOQ	NA
		21.0	20.8 ± 0.1	−0.8
		41.9	42.0 ± 0.8	0.2
		83.8	82.7 ± 1.9	−1.4
		168	162 ± 3	−3.6
		335	329 ± 10	−1.9
April 28, 2016 (Drinking Water Bottle)	June 8, 2016	0	BLOQ	NA
		21.0	22.2 ± 0.2	5.6

Date Prepared	Date Analyzed[a]	Target Concentration (mg/L)	Determined Concentration (mg/L)[b]	Difference from Target (%)
		41.9	44.2 ± 0.8	5.5
		83.8	87.7 ± 0.8	4.6
		168	181 ± 7	7.5
		335	340 ± 3	1.6
April 28, 2016 (Carboy)	June 8, 2016	0	BLOQ	NA
		21.0	22.5 ± 0.5	7.3
		41.9	43.9 ± 0.1	4.8
		83.8	88.3 ± 1.2	5.4
		168	178 ± 4	5.7
		335	355 ± 8	5.9
May 31, 2016 (Drinking Water Bottle)	July 15, 2016	0	BLOQ	NA
		21.0	19.6 ± 0.1	−6.8
		41.9	41.5 ± 0.6	−0.9
		83.8	82.8 ± 0.3	−1.2
		168	163 ± 3	−2.8
		335	339 ± 4	1.3
May 31, 2016 (Carboy)	July 15, 2016	0	BLOQ	NA
		21.0	20.4 ± 0.3	−2.9
		41.9	42.2 ± 0.2	0.7
		83.8	83.7 ± 0.3	−0.2
		168	169 ± 6	0.4
		335	338 ± 5	0.8

BLOQ = below the limit of quantification; NA = not applicable.
[a]Date first chromatograms were acquired.
[b]Data are presented as mean ± standard deviation of triplicate analysis.

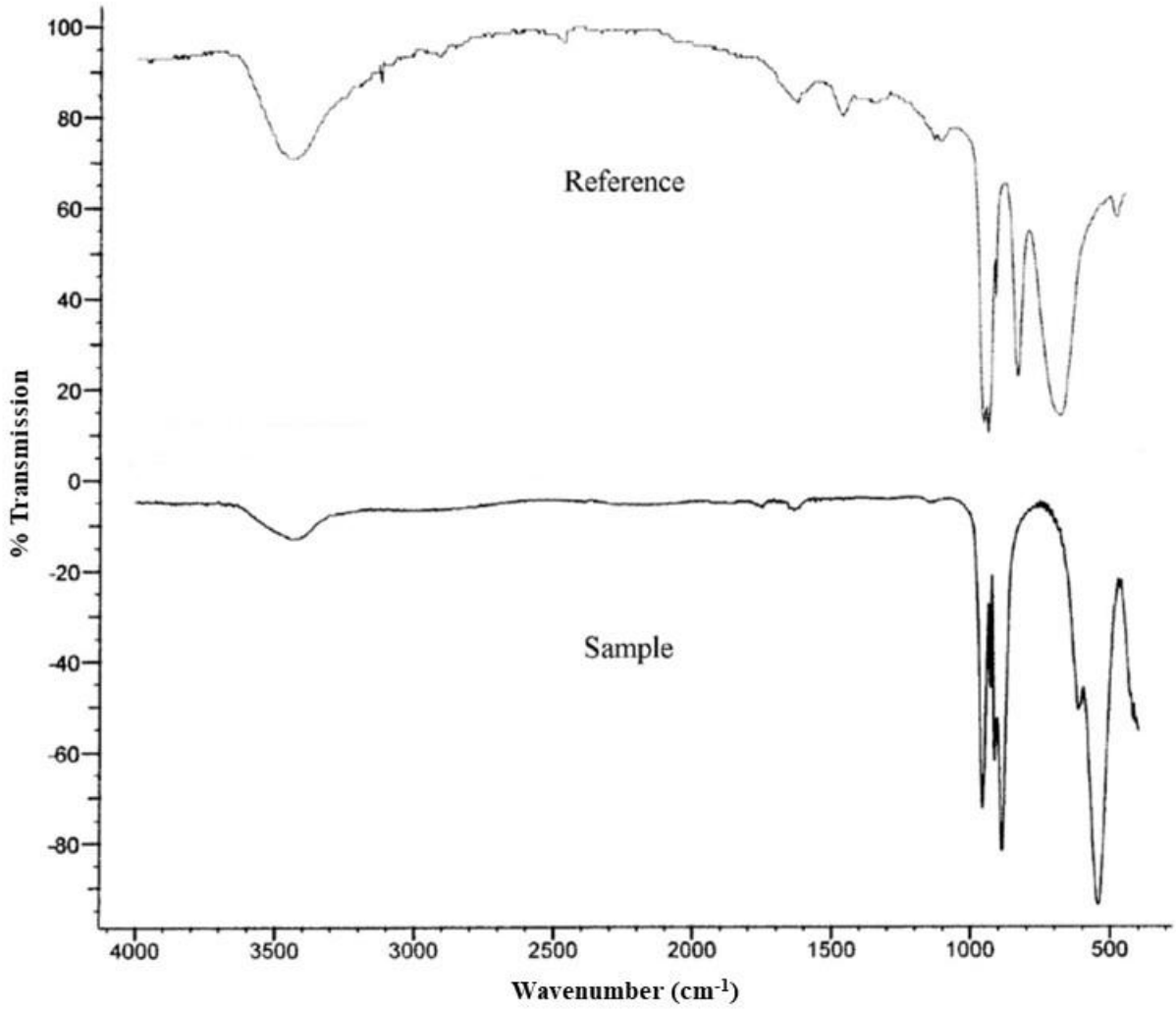

Figure A-1. Infrared Absorption Spectrum of Sodium Metavanadate

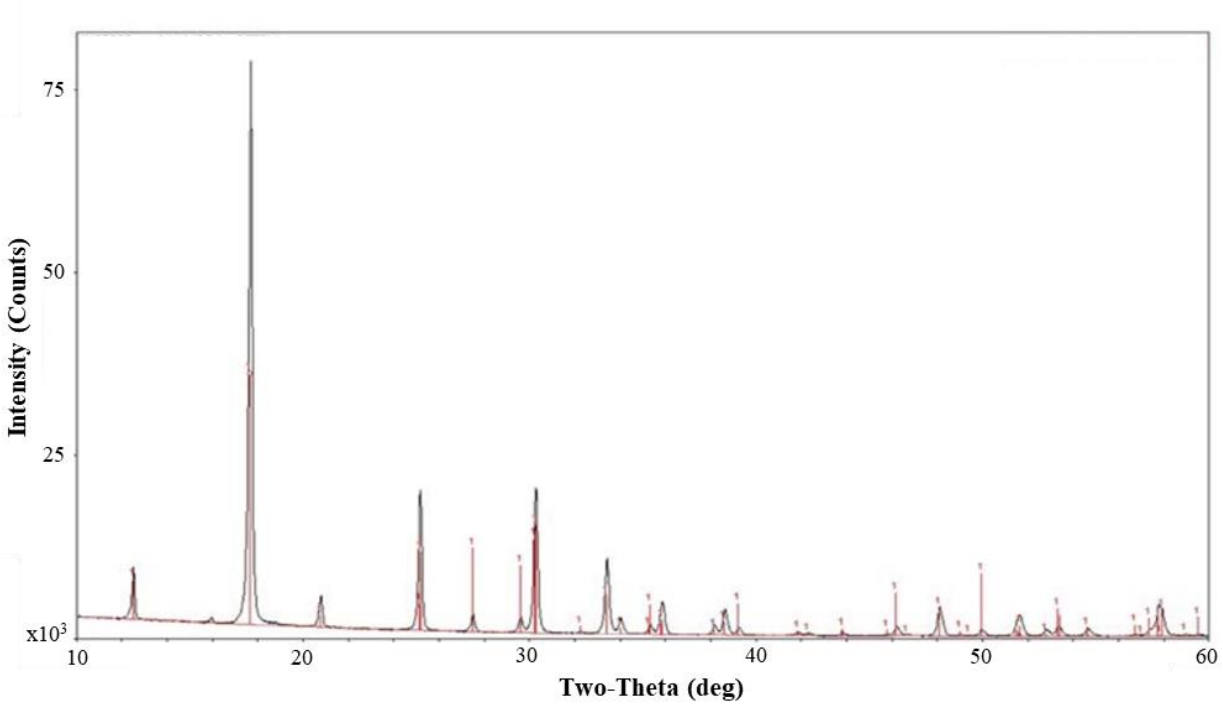

Figure A-2. Indexed X-ray Diffraction Pattern for Sodium Metavanadate and Metamunirite

Indexed X-ray diffraction pattern for the test article sodium metavanadate (black) overlaid with reference pattern (red) of metamunirite ($NaVO_3$). Peaks marked with a 1 indicate a match between the sample and metamunirite.

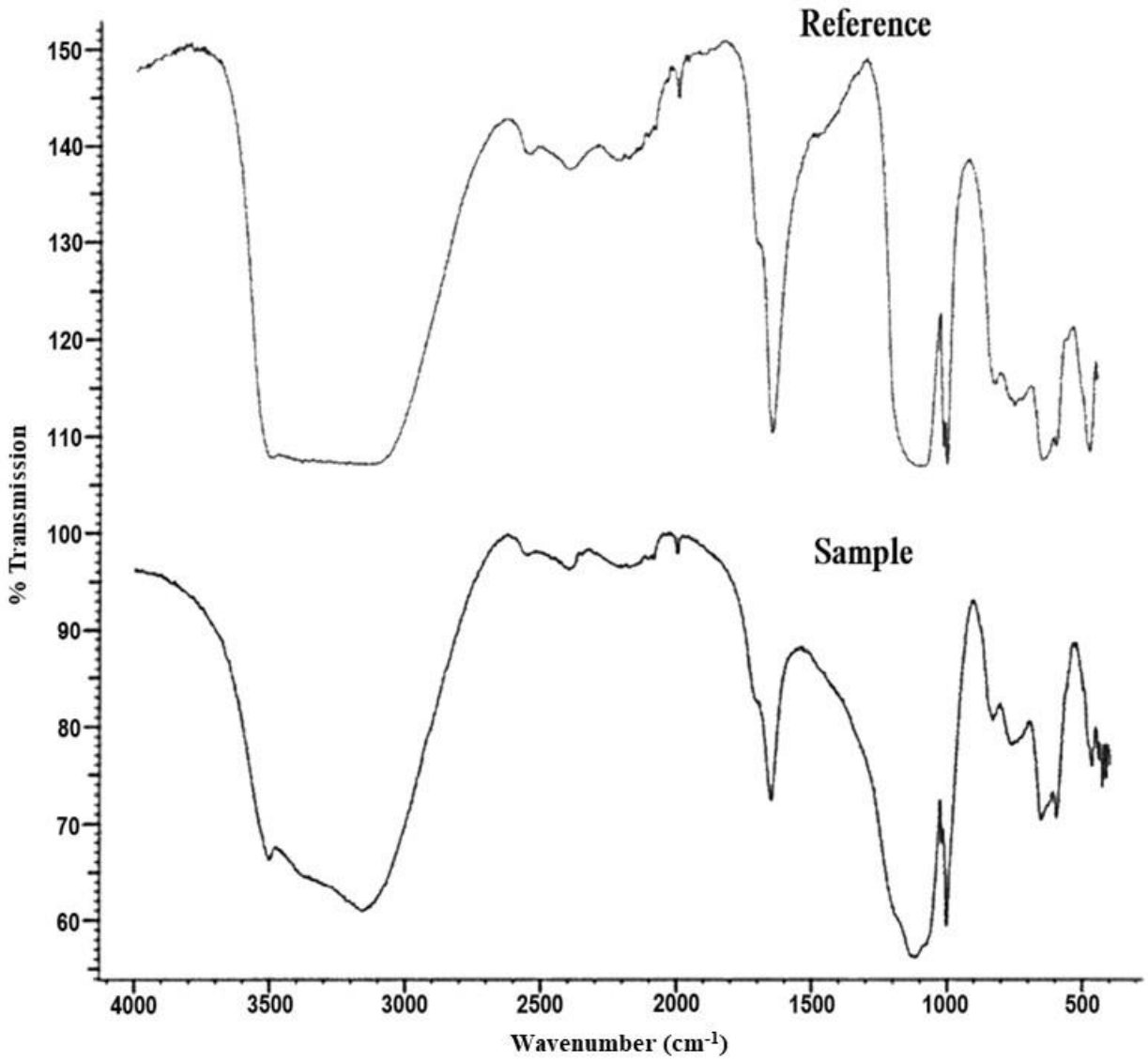

Figure A-3. Infrared Absorption Spectrum of Vanadyl Sulfate

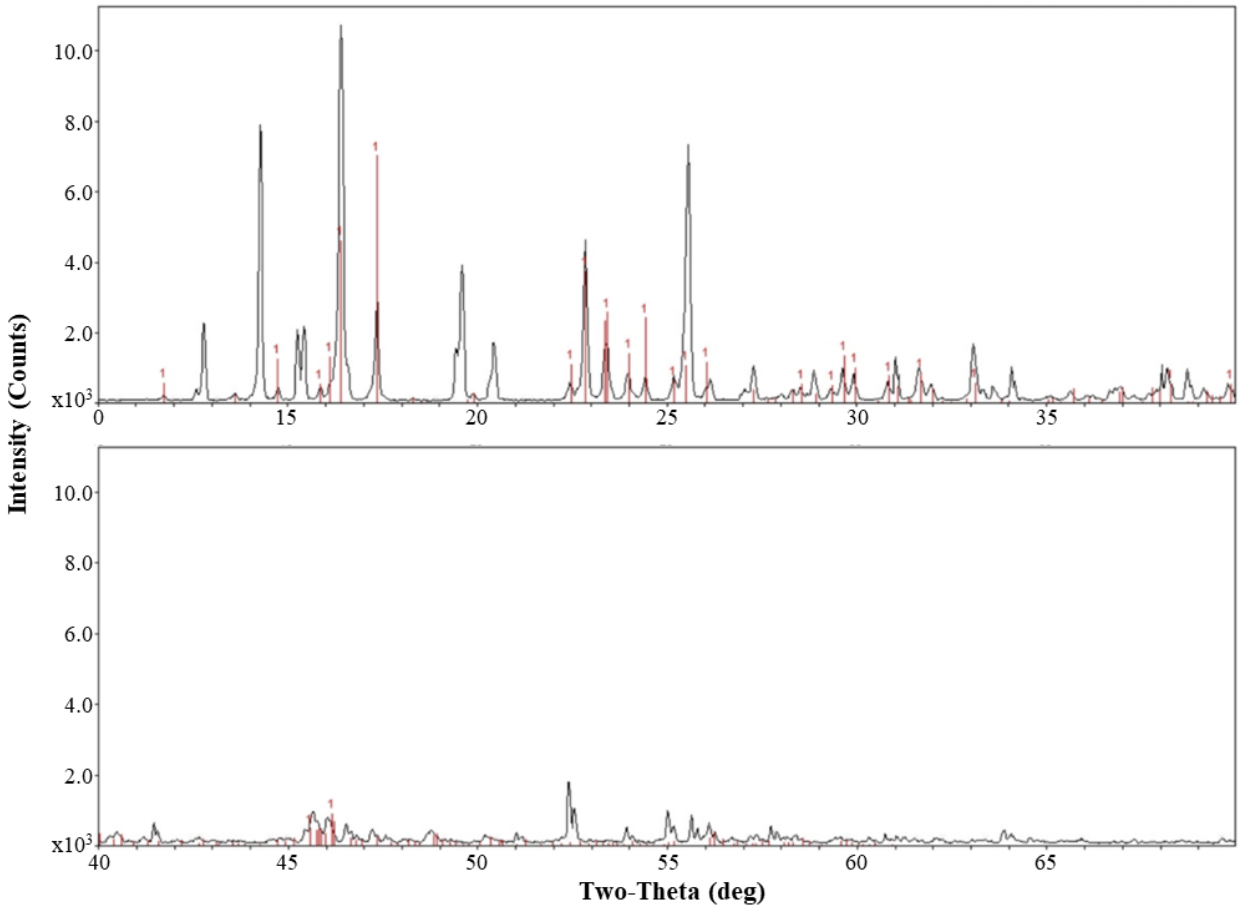

Figure A-4. Indexed X-ray Diffraction Pattern for Vanadyl Sulfate and Minasragrite

Indexed X-ray diffraction pattern for the test article vanadyl sulfate (black) overlaid with the reference pattern (red) of minasragrite ($(VO)(SO_4)\cdot 5H_2O$). Peaks marked with a 1 indicate a match between the sample and minasragrite. The x-axis has been split into two ranges for clarity.

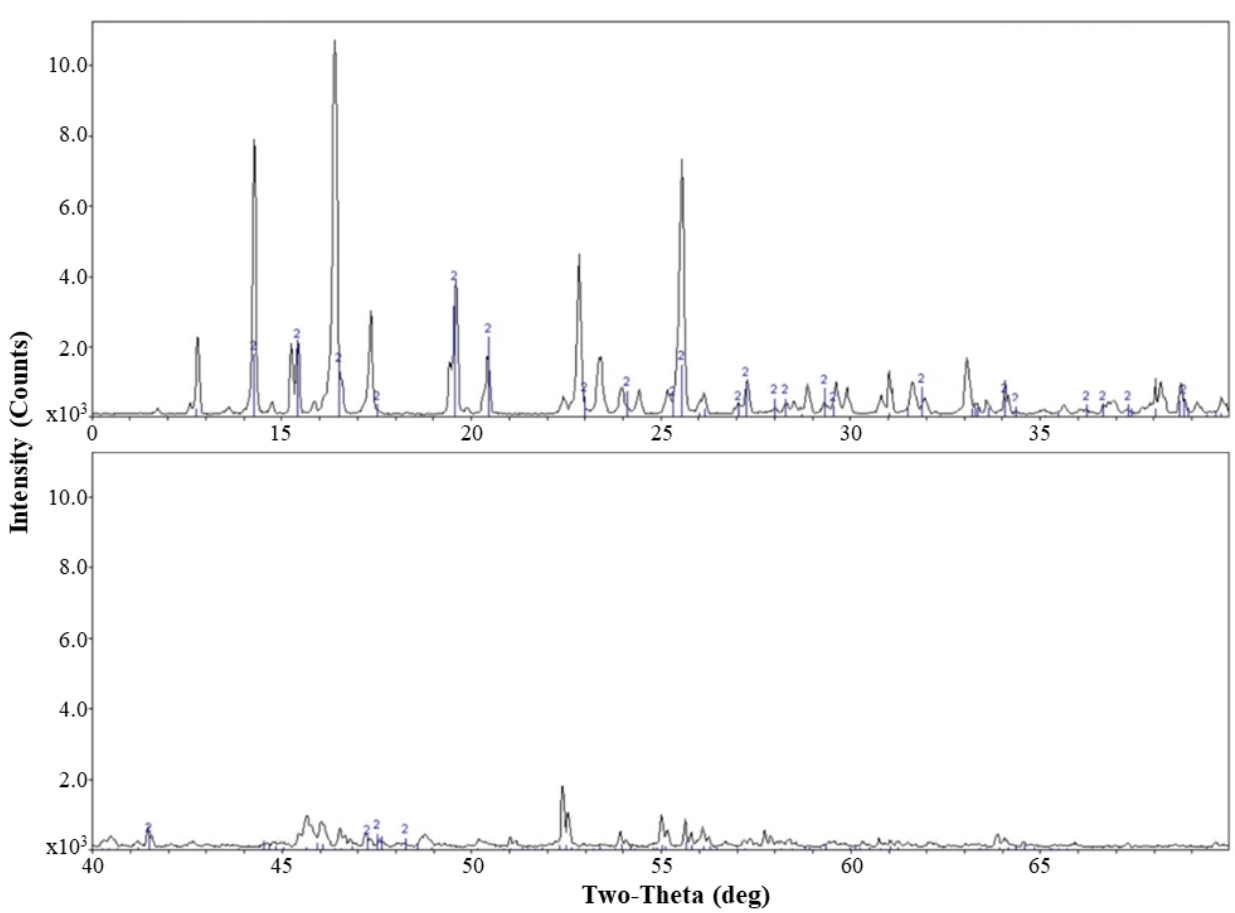

Figure A-5. Indexed X-ray Diffraction Pattern for Vanadyl Sulfate and Bobjonesite

Indexed X-ray diffraction pattern for the test article vanadyl sulfate (black) overlaid with reference pattern (blue) of bobjonesite ($(VO)(SO_4)\cdot 3H_2O$). Peaks marked with a 2 indicate a match between the sample and bobjonesite. The x-axis has been split into two ranges for clarity.

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

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Tables

B.1. NIH-07 Feed

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

Ingredients	Percent by Weight
Ground Hard Winter Wheat	23.0
Ground #2 Yellow Shelled Corn	24.25
Wheat Middlings	10.0
Oat Hulls	0.0
Alfalfa Meal (Dehydrated, 17% Protein)	4.0
Purified Cellulose	0.0
Soybean Meal (47% Protein)	12.0
Fish Meal (62% Protein)	10.0
Corn Oil (without Preservatives)	0.0
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	5.0
Dried Molasses	1.5
Corn Gluten Meal (60% Protein)	3.0
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	–

	Amount[a]	Source
d-Pantothenic Acid	19.8 mg	d-Calcium pantothenate
Riboflavin	3.8 mg	–
Thiamine	11 mg	Thiamine mononitrate
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 diet.

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

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by Weight)	22.87 ± 0.208	22.7–23.1	3
Crude Fat (% by Weight)	5.2 ± 0.0	5.4–5.2	3
Crude Fiber (% by Weight)	2.98 ± 0.437	2.48–3.27	3
Ash (% by Weight)	6.41 ± 0.086	6.32–6.49	3
Amino Acids (% of Total Diet)			
Arginine	1.278 ± 0.343	0.258–1.49	11
Cystine	0.307 ± 0.059	0.153–0.372	11
Glycine	1.065 ± 0.289	0.217–1.31	11
Histidine	0.482 ± 0.121	0.125–0.553	11
Isoleucine	0.914 ± 0.233	0.214–1.03	11
Leucine	1.873 ± 0.485	0.423–2.13	11
Lysine	1.140 ± 0.345	0.111–1.32	11
Methionine	0.453 ± 0.117	0.102–0.515	11
Phenylalanine	1.023 ± 0.245	0.286–1.12	11
Threonine	0.850 ± 0.228	0.168–0.961	11
Tryptophan	0.259 ± 0.064	0.076–0.326	11
Tyrosine	0.801 ± 0.200	0.209–0.894	11
Valine	1.055 ± 0.264	0.262–1.17	11

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Essential Fatty Acids (% of Total Diet)			
Linoleic	2.436 ± 0.489	0.199–3.77	11
Linolenic	0.367 ± 0.397	0.214–1.56	11
Vitamins			
Vitamin A (IU/kg)	4,180 ± 46.03	3,730–4,650	3
α-Tocopherol (ppm)	6,097 ± 20,067	31.36–66,600	11
Thiamine (ppm)[a]	14.3 ± 1.473	12.6–15.2	3
Riboflavin (ppm)	13.54 ± 4.438	4.2–19.8	11
Niacin (ppm)	95.02 ± 16.30	51.9–112.0	11
Pantothenic Acid (ppm)	40.69 ± 12.76	3.8–51.1	11
Pyridoxine (ppm)[a]	11.74 ± 4.81	0.42–19.7	11
Folic Acid (ppm)	2.38 ± 0.571	1.37–3.09	11
Biotin (ppm)	0.300 ± 0.187	0.0–0.638	11
B_{12} (ppb)	45.27 ± 15.14	4.0–61.6	11
Choline (as Chloride) (ppm)	1,719.0 ± 386.0	700.0–2,200.0	11
Minerals			
Calcium (%)	1.14 ± 0.055	1.09–1.2	3
Phosphorus (%)	0.94 ± 0.069	0.862–0.992	3
Potassium (%)	0.762 ± 0.226	0.088–0.88	11
Chloride (%)	0.656 ± 0.102	0.411–0.8	11
Sodium (%)	0.409 ± 0.112	0.318–0.721	11
Magnesium (%)	0.171 ± 0.053	0.0162–0.218	11
Iron (ppm)	353.3 ± 117.5	35.7–469.0	11
Manganese (ppm)	82.88 ± 27.28	3.53–104.0	11
Zinc (ppm)	58.75 ± 20.3	4.74–89.2	11
Copper (ppm)	12.91 ± 4.73	0.683–21.1	11
Iodine (ppm)	1.647 ± 1.088	0.0–3.45	11
Chromium (ppm)	3.95 ± 0.035	3.89–4.0	9
Cobalt (ppm)	0.470 ± 0.296	0.01–0.963	11

[a]As hydrochloride.

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

	Mean ± Standard Deviation	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.481 ± 0.006	0.474–0.486	3
Cadmium (ppm)	0.087 ± 0.004	0.083–0.09	3
Lead (ppm)	0.084 ± 0.007	0.077–0.091	3
Mercury (ppm)	0.017 ± 0.003	0.014–0.02	3
Selenium (ppm)	0.286 ± 0.020	0.264–0.304	3
Aflatoxins (ppb)[a]	<5.0	–	3
Nitrate Nitrogen (ppm)[b]	9.54 ± 1.11	8.71–10.8	3
Nitrite Nitrogen (ppm)[a,b]	<0.61	–	3
BHA (ppm)[a,c]	<1.0	–	3
BHT (ppm)[a,c]	<1.0	–	3
Aerobic Plate Count (CFU/g)	<10.0	–	3
Coliform (MPN/g)	<3.0	–	3
Escherichia coli (MPN/g)[a]	<3.0	–	3
Salmonella sp. (MPN/g)	Negative	–	3
Total Nitrosamines (ppb)[d]	9.67 ± 2.53	6.8–11.6	3
N-Nitrosodimethylamine (ppb)[d]	6.95 ± 0.919	6.3–7.6	3
N-Nitrosopyrrolidine (ppb)[d]	5.03 ± 1.54	4.0–6.8	3
Pesticides (ppm)			
α-BHC[a]	<0.01	–	3
β-BHC[a]	<0.02	–	3
γ-BHC[a]	<0.01	–	3
δ-BHC[a]	<0.01	–	3
Heptachlor[a]	<0.01	–	3
Aldrin[a]	<0.01	–	3
Heptachlor Epoxide[a]	<0.01	–	3
DDE[a]	<0.01	–	3
DDD[a]	<0.01	–	3
DDT[a]	<0.01	–	3
HCB[a]	<0.01	–	3
Mirex[a]	<0.01	–	3
Methoxychlor[a]	<0.05	–	3
Dieldrin[a]	<0.01	–	3
Endrin[a]	<0.01	–	3

	Mean ± Standard Deviation	Range	Number of Samples
Telodrin[a]	<0.01	–	3
Chlordane[a]	<0.05	–	3
Toxaphene[a]	<0.10	–	3
Estimated PCBs[a]	<0.20	–	3
Ronnel[a]	<0.01	–	3
Ethion[a]	<0.02	–	3
Trithion[a]	<0.05	–	3
Diazinon[a]	<0.10	–	3
Methyl Chlorpyrifos	0.071 ± 0.077	0.02–0.16	3
Methyl Parathion[a]	<0.02	–	3
Ethyl Parathion[a]	<0.02	–	3
Malathion	0.0835 ± 0.055	0.0285–0.138	3
Endosulfan I[a]	<0.01	–	3
Endosulfan II[a]	<0.01	–	3
Endosulfane Sulfate[a]	<0.03	–	3

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	23.00
Ground #2 Yellow Shelled Corn	22.44
Wheat Middlings	15.0
Oat Hulls	8.5
Alfalfa Meal (Dehydrated, 17% Protein)	7.5
Purified Cellulose	5.5
Soybean Meal (49% Protein)	4.0
Fish Meal (60% Protein)	4.0
Corn Oil (without Preservatives)	3.0
Soy Oil (without Preservatives)	3.0

Ingredients	Percent by Weight
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
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	α-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 diet.

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

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by Weight)	14.7 ± 0.454	14.0–15.3	11
Crude Fat (% by Weight)	8.25 ± 0.202	7.9–8.5	11
Crude Fiber (% by Weight)	9.54 ± 0.304	9.08–10.1	11
Ash (% by Weight)	4.95 ± 0.101	4.77–5.09	11
Amino Acids (% of Total Diet)			
Arginine	0.806 ± 0.073	0.67–0.97	31
Cystine	0.220 ± 0.021	0.15–0.25	31
Glycine	0.702 ± 0.037	0.62–0.8	31
Histidine	0.341 ± 0.068	0.27–0.68	31
Isoleucine	0.548 ± 0.039	0.43–0.66	31
Leucine	1.095 ± 0.061	0.96–1.24	31
Lysine	0.070 ± 0.101	0.31–0.86	31
Methionine	0.409 ± 0.040	0.26–0.49	31
Phenylalanine	0.623 ± 0.045	0.471–0.72	31
Threonine	0.513 ± 0.040	0.43–0.61	31
Tryptophan	0.156 ± 0.026	0.11–0.2	31
Tyrosine	0.425 ± 0.064	0.28–0.54	31
Valine	0.666 ± 0.038	0.55–0.73	31
Essential Fatty Acids (% of Total Diet)			
Linoleic	3.936 ± 0.229	3.49–4.55	31
Linolenic	0.306 ± 0.030	0.21–0.368	31
Vitamins			
Vitamin A (IU/kg)	3,060 ± 101.1	2,090–5,130	11
α-Tocopherol (ppm)	2,300 ± 12,398	13.6–69,100	31
Thiamine (ppm)[a]	7.65 ± 0.530	7.0–8.6	11
Riboflavin (ppm)	8.19 ± 2.747	4.2–17.5	31
Niacin (ppm)	80.10 ± 9.733	66.4–107.0	31
Pantothenic Acid (ppm)	26.28 ± 10.69	17.4–81.0	31
Pyridoxine (ppm)[a]	9.657 ± 2.013	6.44–14.3	31
Folic Acid (ppm)	1.59 ± 0.434	1.15–3.27	31
Biotin (ppm)	0.331 ± 0.096	0.2–0.704	31
B_{12} (ppb)	50.15 ± 33.77	18.3–174.0	31
Choline (as Chloride) (ppm)	2,553 ± 632	1,160–3,790	31

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Minerals			
Calcium (%)	0.890 ± 0.051	0.832–0.976	11
Phosphorus (%)	0.558 ± 0.023	0.522–0.596	11
Potassium (%)	0.666 ± 0.034	0.563–0.733	31
Chloride (%)	0.389 ± 0.044	0.3–0.517	31
Sodium (%)	0.193 ± 0.027	0.153–0.283	31
Magnesium (%)	0.217 ± 0.053	0.185–0.49	31
Iron (ppm)	190.0 ± 35.69	135–311	31
Manganese (ppm)	49.95 ± 9.12	21.0–73.1	31
Zinc (ppm)	56.51 ± 24.88	42.5–184	31
Copper (ppm)	7.61 ± 2.42	3.21–16.3	31
Iodine (ppm)	0.530 ± 0.230	0.0–1.0	31
Chromium (ppm)	1.213 ± 1.25	0.33–3.97	31
Cobalt (ppm)	0.216 ± 0.148	0.086–0.864	29

[a]As hydrochloride.

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

	Mean ± Standard Deviation	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.241 ± 0.024	0.211–0.287	11
Cadmium (ppm)	0.051 ± 0.003	0.045–0.056	11
Lead (ppm)	0.093 ± 0.031	0.066–0.158	11
Mercury (ppm)	0.010 ± 0.0	0.009–0.01	11
Selenium (ppm)	0.163 ± 0.023	0.134–0.201	11
Aflatoxins (ppb)[a]	<5.0	–	11
Nitrate Nitrogen (ppm)[b]	11.67 ± 0.023	9.02–16.58	11
Nitrite Nitrogen (ppm)[a,b]	<0.61	–	11
BHA (ppm)[a,c]	<1.0	–	11
BHT (ppm)[a,c]	<1.0	–	11
Aerobic Plate Count (CFU/g)	25.45 ± 51.26	10.0–180.0	11
Coliform (MPN/g)	<3.0	–	11
Escherichia coli (MPN/g)[a]	<3.0	–	11
Salmonella sp. (MPN/g)	Negative	–	11
Total Nitrosamines (ppb)[d]	8.4 ± 3.8	2.4–14.2	11
N-Nitrosodimethylamine (ppb)[d]	2.9 ± 1.9	1.0–5.0	11
N-Nitrosopyrrolidine (ppb)[d]	5.5 ± 2.8	1.2–9.5	11

	Mean ± Standard Deviation	Range	Number of Samples
Pesticides (ppm)			
α-BHC[a]	<0.01	–	11
β-BHC[a]	<0.02	–	11
γ-BHC[a]	<0.01	–	11
δ-BHC[a]	<0.01	–	11
Heptachlor[a]	<0.01	–	11
Aldrin[a]	<0.01	–	11
Heptachlor Epoxide[a]	<0.01	–	11
DDE[a]	<0.01	–	11
DDD[a]	<0.01	–	11
DDT[a]	<0.01	–	11
HCB[a]	<0.01	–	11
Mirex[a]	<0.01	–	11
Methoxychlor[a]	<0.05	–	11
Dieldrin[a]	<0.01	–	11
Endrin[a]	<0.01	–	11
Telodrin[a]	<0.01	–	11
Chlordane[a]	<0.05	–	11
Toxaphene[a]	<0.01	–	11
Estimated PCBs[a]	<0.20	–	11
Ronnel[a]	<0.01	–	11
Ethion[a]	<0.02	–	11
Trithion[a]	<0.05	–	11
Diazinon[a]	<0.10	–	11
Methyl Chlorpyrifos	0.172 ± 0.105	0.0–0.36	11
Ethyl Chlorpyrifos	0.009 ± 0.013	0.0–0.025	11
Methyl Pirimiphos	0.013 ± 0.017	0.0–0.05	11
Methyl Parathion[a]	<0.02	–	11
Ethyl Parathion[a]	<0.02	–	11
Malathion	0.180 ± 0.147	0.02–0.585	11
Endosulfan I[a]	<0.01	–	11
Endosulfan II[a]	<0.01	–	11
Endosulfane Sulfate[a]	<0.03	–	11

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.

Appendix C. Sentinel Animal Program

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Tables

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 toxicity studies, blood samples were collected from each sentinel animal and 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 testing performed in-house or by 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 below; the times at which samples were collected during the studies are also listed (Table C-1, Table C-2, Table C-3, Table C-4).

C.2. Results

Rats: All 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 in the Three-month Study of Sodium Metavanadate

Three-month Study			
Collection Timepoints	**Quarantine[a]**	**4 Weeks[b]**	**Study Termination**
Number Examined (Males/Females)	0/10	5/5	5/5
Method/Test			
Multiplex Fluorescent Immunoassay (MFI)			
Kilham rat virus (KRV)	–	–	–
Mycoplasma pulmonis	–	–	–
Pneumocystis carinii	NT	NT	–
Pneumonia virus of mice (PVM)	–	–	–
Rat coronavirus/sialodacryoadenitis virus (RCV/SDA)	–	–	–
Rat minute virus (RMV)	–	–	–
Rat parvo virus (RPV)	–	–	–

Three-month Study			
Rat theilovirus (RTV)	–	–	–
Sendai	–	–	–
Theiler's murine encephalomyelitis virus (TMEV)	–	–	–
Toolan's H-1	–	–	–
In-house Evaluation			
Parasite evaluation (evaluation of cecal contents and perianal surface)	–	–	NT

– = negative; NT = not tested.
[a]Age matched nonpregnant females.
[b]F_1 sentinel animals tested 4 weeks after study start.

Table C-2. Methods and Results for Sentinel Animal Testing in Male and Female Mice in the Three-month Study of Sodium Metavanadate

Three-month Study			
Collection Timepoints	**Quarantine**	**4 Weeks**	**Study Termination**
Number Examined (Males/Females)	5/5	5/5	5/5
Method/Test			
Multiplex Fluorescent Immunoassay (MFI)			
Ectromelia virus	–	–	–
Epizootic diarrhea of infant mice (EDIM)	–	–	–
Lymphocytic choriomeningitis virus (LCMV)	–	–	–
Mycoplasma pulmonis	–	–	–
Mouse hepatitis virus (MHV)	–	–	–
Mouse norovirus (MNV)	–	–	–
Mouse parvovirus (MPV)	–	–	–
Minute virus of mice (MVM)	–	–	–
Pneumonia virus of mice (PVM)	–	–	–
Reovirus (REO3)	–	–	–
Rat theilovirus (RTV)	–	–	–
Sendai	–	–	–
Theiler's murine encephalomyelitis virus (TMEV) GDVII	–	–	–
In-house Evaluation			
Parasite evaluation (evaluation of cecal contents and perianal surface)	–	–	NT

– = negative; NT = not tested.

Table C-3. Methods and Results for Sentinel Animal Testing in Male and Female Rats in the Three-month Study of Vanadyl Sulfate

Three-month Study			
Collection Timepoints	**Quarantine[a]**	**4 Weeks[b]**	**Study Termination**
Number Examined (Males/Females)	0/10	5/5	5/5
Method/Test			
Multiplex Fluorescent Immunoassay (MFI)			
Kilham rat virus (KRV)	–	–	–
Mycoplasma pulmonis	–	–	–
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)	–	–	–
Toolan's H-1	–	–	–
In-house Evaluation			
Parasite evaluation (evaluation of cecal contents and perianal surface)	–	–	NT

– = negative; NT = not tested.
[a]Age matched nonpregnant females.
[b]F_1 sentinel animals tested 4 weeks after study start.

Table C-4. Methods and Results for Sentinel Animal Testing in Male and Female Mice in the Three-month Study of Vanadyl Sulfate

Three-month Study				
Collection Timepoints	**Quarantine**	**4 Weeks**	**7 Weeks**	**Study Termination**
Number Examined (Males/Females)	5/5	5/5	1/0	5/5
Method/Test				
Multiplex Fluorescent Immunoassay (MFI)				
Ectromelia virus	–	–	–	–
Epizootic diarrhea of infant mice (EDIM)	–	–	–	–
Lymphocytic choriomeningitis virus (LCMV)	–	–	–	–
Mycoplasma pulmonis	–	–	–	–
Mouse hepatitis virus (MHV)	–	–	–	–
Mouse norovirus (MNV)	–	–	–	–
Mouse parvovirus (MPV)	–	–	–	–

Three-month Study				
Minute virus of mice (MVM)	–	–	–	–
Pneumonia virus of mice (PVM)	–	–	–	–
Reovirus (REO3)	–	–	–	–
Rat theilovirus (RTV)	–	–	–	–
Sendai	–	–	–	–
Theiler's murine encephalomyelitis virus (TMEV) GDVII	–	–	–	–
In-house Evaluation				
Parasite evaluation (evaluation of cecal contents and perianal surface)	–	–	NT	NT

– = negative; NT = not tested.

Appendix D. Genetic Toxicology

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Tables

D.1. Evaluation Protocol

The National Toxicology Program (NTP) considers biological as well as statistical factors to determine an overall assay result. For an individual assay, the statistical procedures for data analysis are described in the following 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. In such cases, all the data are critically evaluated with attention given to possible protocol variations in determining the weight of evidence for an overall conclusion of chemical activity in an assay. For in vitro assays conducted with and without exogenous metabolic activation, results obtained in the absence of activation are analyzed separately from results obtained in the presence of activation. The summary table in the abstract of this toxicity report presents the Division of Translational Toxicology's (DTT's) scientific judgment regarding the overall evidence for activity of the chemical in an assay.

D.2. Bacterial Mutagenicity

D.2.1. Bacterial Mutagenicity Test Protocol

Testing procedures were modified from those originally reported by Zeiger et al.[94] Coded samples of sodium metavanadate and vanadyl sulfate (the same chemical lots that were used in the perinatal and 3-month 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 after incubation for 2 days at 37°C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and at least six doses of sodium metavanadate or vanadyl sulfate. The highest concentration of sodium metavanadate and vanadyl sulfate tested was limited by toxicity in strains TA98 and TA100; *E. coli* was 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- or tryptophan-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants that is not dose-related, is not reproducible, or is not of sufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed after 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.2.2. Results

Neither sodium metavanadate nor vanadyl sulfate was mutagenic in *Salmonella typhimurium* strains TA98 and TA100 or in *Escherichia coli* strain WP2 *uvrA* (pKM101) in tests conducted with and without exogenous metabolic activation provided by phenobarbital/benzoflavone-

induced male rat liver S9 and cofactors (Table D-1, Table D-2). Sodium metavanadate was tested at concentrations of 0.5–100 µg/plate in TA98 with or without S9 mix and in TA100 at concentrations of 0.5–100 µg/plate without S9 mix and 1.0–500 µg/plate with S9 mix. For TA98 and TA100, the top concentration was limited by cytotoxicity. In *E. coli*, sodium metavanadate was tested at concentrations of 40–6,000 µg/plate with or without S9 mix. Vanadyl sulfate was tested at concentrations of 0.4–100 µg/plate in TA98 and TA100 with or without S9 mix. For TA98, the top concentration was limited by cytotoxicity. In *E. coli*, vanadyl sulfate was tested at concentrations of 4.0–6,000 µg/plate with or without S9 mix.

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

Strain	Concentration (µg/plate)	Without S9	Without S9	With 10% Rat S9	With 10% Rat S9
TA98					
	0	19 ± 1.0	21 ± 1.5	24 ± 7.6	34 ± 1.7
	0.5	21 ± 3.8	21 ± 1.7	30 ± 0.7	27 ± 3.2
	1.0	18 ± 3.0	22 ± 2.6	23 ± 3.0	26 ± 1.2
	2.5	18 ± 2.5	22 ± 1.2	24 ± 6.4	24 ± 1.2
	10.0	12 ± 2.5	18 ± 3.8	29 ± 3.2	27 ± 2.3
	20.0	–	Toxic	–	–
	40.0	Toxic	Toxic	15 ± 2.8	9 ± 1.5
	100.0	Toxic	–	Toxic	Toxic
Trial Summary		Negative	Negative	Negative	Negative
Positive Control[b]		547 ± 39.3	377 ± 13.5	1,330 ± 58.9	1,477 ± 35.6
TA100					
	0	105 ± 5.4	108 ± 1.5	95 ± 4.7	113 ± 2.6
	0.5	98 ± 9.7	120 ± 3	–	–
	1.0	108 ± 8.2	111 ± 3.2	–	105 ± 9.6
	2.5	101 ± 9.2	120 ± 1.5	97 ± 8.9	120 ± 4.3
	10.0	125 ± 4.9	117 ± 1.5	90 ± 2	98 ± 18.8
	20.0	–	113 ± 6.5	–	–
	40.0	22 ± 8.1	33 ± 8.5	99 ± 8.7	97 ± 6.6
	100.0	8 ± 2	–	109 ± 3.2	97 ± 13.5
	200.0	–	–	Toxic	Toxic
	500.0	–	–	Toxic	–
Trial Summary		Negative	Negative	Negative	Negative
Positive Control		772 ± 18.2	887 ± 32.3	1,594 ± 195.9	2,165 ± 73.1
***Escherichia coli* WP2 *uvrA* (pKM101)**					
	0	150 ± 7.0	161 ± 5.5	181 ± 11.1	150 ± 7
	40.0	–	143 ± 2.3	–	166 ± 9.8

Strain	Concentration (µg/plate)	Without S9	Without S9	With 10% Rat S9	With 10% Rat S9
	100.0	141 ± 9.1	149 ± 0	152 ± 3.4	146 ± 7.2
	200.0	153 ± 14.9	133 ± 4	169 ± 2.9	146 ± 8.5
	500.0	131 ± 3.5	121 ± 10.8	156 ± 3.8	149 ± 6.2
	1,000.0	130 ± 11.5	–	151 ± 18.1	–
	2,000.0	–	116 ± 11.4	–	133 ± 18.4
	3,000.0	112 ± 4.9	–	138 ± 12.2	–
	6,000.0	120 ± 5.5	133 ± 3.8	140 ± 6.4	140 ± 2.1
Trial Summary		Negative	Negative	Negative	Negative
Positive Control		2,697 ± 29.4	612 ± 59.6	1,088 ± 18.5	1,051 ± 66.2

[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 (distilled water).
[b]The positive controls in the absence of metabolic activation were 2-nitrofluorene (TA98), sodium azide (TA100), and methyl methane sulfonate (*E. coli*). The positive control for metabolic activation with all strains was 2-aminoanthracene.

Table D-2. Mutagenicity of Vanadyl Sulfate in Bacterial Tester Strains[a]

Strain	Concentration (µg/plate)	Without S9	Without S9	With 10% Rat S9	With 10% Rat S9
TA98					
	0	26 ± 4.6	18 ± 1.7	28 ± 4.0	27 ± 3.0
	0.4	21 ± 1.8	20 ± 2.3	30 ± 1.8	24 ± 3.3
	1.0	24 ± 1.5	21 ± 3.3	24 ± 1.9	30 ± 2.2
	4.0	24 ± 2.6	18 ± 4.3	28 ± 2.9	25 ± 3.0
	10.0	16 ± 3.1	19 ± 1.5	24 ± 3.2	30 ± 2.0
	40.0	9 ± 2.5	6 ± 3.2	27 ± 4.3	32 ± 3.2
	100.0	5 ± 1.9	Toxic	14 ± 2.2	12 ± 1.5
Trial Summary		Negative	Negative	Negative	Negative
Positive Control[b]		591 ± 17.3	608 ± 103.6	1,576 ± 34.5	1,872 ± 57.8
TA100					
	0	103 ± 7.1	113 ± 4.9	98 ± 5.7	104 ± 9.3
	0.4	102 ± 8.8	98 ± 10.9	105 ± 2.6	113 ± 3.4
	1.0	115 ± 16.8	101 ± 5.0	92 ± 1.5	99 ± 9.6
	4.0	88 ± 7.6	100 ± 4.4	92 ± 5.5	111 ± 5.4
	10.0	103 ± 5.0	114 ± 4.4	99 ± 5.7	125 ± 5.5
	40.0	100 ± 10.4	145 ± 6.7	88 ± 10.1	89 ± 2.0
	100.0	30 ± 5.2	59 ± 4.1	83 ± 3.5	101 ± 4.8
Trial Summary		Negative	Negative	Negative	Negative
Positive Control		693 ± 19.6	699 ± 75.0	3,780 ± 78.9	2,306 ± 28.6

Strain	Concentration (µg/plate)	Without S9	Without S9	With 10% Rat S9	With 10% Rat S9
***Escherichia coli* WP2 *uvrA* (pKM101)**					
	0	159 ± 6.3	159 ± 11.6	177 ± 2.1	178 ± 9.0
	4.0	122 ± 9.5	–	169 ± 10.5	–
	10.0	141 ± 7.2	–	170 ± 9.0	–
	40.0	163 ± 8.4	145 ± 5.3	148 ± 10.1	179 ± 11.6
	100.0	134 ± 6.7	134 ± 5.2	149 ± 7.7	175 ± 6.8
	400.0	132 ± 8.2	141 ± 7.8	161 ± 8.7	149 ± 12.5
	1,000.0	151 ± 4.1	143 ± 14.7	155 ± 4.1	157 ± 4.4
	3,000.0	–	139 ± 10.1	–	166 ± 6.6
	6,000.0	–	148 ± 3.5	–	198 ± 5.5[c]
Trial Summary		Negative	Negative	Negative	Negative
Positive Control		2,192 ± 96.1	1,047 ± 78.2	1,310 ± 26.8	1,070 ± 30.1

[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 (distilled water).
[b]The positive controls in the absence of metabolic activation were 2-nitrofluorene (TA98), sodium azide (TA100), and methyl methane sulfonate (*E. coli*). The positive control for metabolic activation with all strains was 2-aminoanthracene.
[c]Precipitate on plate.

D.3. Micronucleus Assay

D.3.1. Peripheral Blood Micronucleus Test Protocol

At termination of the 3-month toxicity studies of sodium metavanadate and vanadyl sulfate, 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 (i.e., reticulocytes or polychromatic erythrocytes [PCEs]) and mature erythrocytes (i.e., normochromatic erythrocytes [NCEs]) using a flow cytometer[95]; both the mature and 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+ 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 PCEs in circulation.[96] In mice, both the mature and immature 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 after 4 weeks of continuous exposure. Approximately 20,000 PCEs and 1×10^6 NCEs 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 chemical exposure.

Prior experience with the large number of cells scored using flow cytometric scoring techniques[97] 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 control group are considered statistically significant at $p \leq 0.025$.

In the micronucleus test, it is preferable to base a positive result on the presence of both a positive trend as well as at least one significantly elevated exposed 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 positive trend or a single significant exposed group generally results in an equivocal call. The absence of both a trend and any significant differences between exposed groups and the control 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.3.2. Results

At the end of the 3-month studies of sodium metavanadate and vanadyl sulfate, peripheral blood samples were obtained from male and female rats and mice and analyzed for the frequency of micronucleated immature erythrocytes (i.e., reticulocytes or polychromatic erythrocytes [PCEs]) and mature erythrocytes (i.e., normochromatic erythrocytes [NCEs]). In male and female rats, the reticulocyte population 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.

In the sodium metavanadate study, a significant trend test was observed for the frequency of micronucleated reticulocytes in male rats, meeting statistical criteria for an equivocal result (Table D-3). However, the frequencies of micronucleated reticulocytes for the control group and all groups exposed to sodium metavanadate were well within the laboratory historical vehicle control (mean ± 2 standard deviations, or 95% confidence interval); therefore, this result was judged to be negative. Significant increases in the percentage of reticulocytes were observed in male rats, indicating that exposure to sodium metavanadate had a stimulatory effect on erythropoiesis. No increases in the frequencies of micronucleated reticulocytes were observed in female rats after 3 months of exposure to sodium metavanadate via drinking water. No increases in the frequencies of micronucleated reticulocytes or micronucleated mature erythrocytes were observed in male or female mice exposed to sodium metavanadate in drinking water for 3 months (Table D-4). However, significant increases in the percentage of reticulocytes were

observed for both male and female mice, indicating that exposure to sodium metavanadate had a stimulatory effect on erythropoiesis.

In the vanadyl sulfate study, male and female rats did not show an increase in micronucleated reticulocytes (Table D-5). No significant changes in the percentage of reticulocytes were observed in male or female rats, suggesting that vanadyl sulfate exposure did not affect erythropoiesis. No significant increases in the frequencies of micronucleated reticulocytes or micronucleated mature erythrocytes were observed in male or female mice administered vanadyl sulfate in drinking water for 3 months (Table D-6). However, significant increases in the percentage of reticulocytes were observed for male and female mice, indicating that exposure to vanadyl sulfate had a stimulatory effect on erythropoiesis.

Table D-3. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Male and Female Rats in the Three-month Drinking Water Study of Sodium Metavanadate[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	0.468 ± 0.049		0.082 ± 0.019		0.934 ± 0.210	
31.3	5	0.440 ± 0.068	1.0000	0.074 ± 0.027	1.0000	1.218 ± 0.046	1.0000
62.5	5	0.550 ± 0.065	1.0000	0.115 ± 0.018	1.0000	1.269 ± 0.080	0.9191
125	5	0.539 ± 0.103	1.0000	0.132 ± 0.018	0.7439	1.274 ± 0.053	0.6569
250	5	0.490 ± 0.090	1.0000	0.198 ± 0.036	0.1015	1.385 ± 0.127	0.0978
500	5	0.750 ± 0.092	0.0751	0.292 ± 0.037	0.0044	1.636 ± 0.082	0.0016
Trend[d]		p = 0.0204		p < 0.001		p < 0.001	
Female							
Exposure Concentration (mg/L)							
0	5	0.620 ± 0.070		0.085 ± 0.010		1.132 ± 0.095	
31.3	5	0.650 ± 0.097	1.0000	0.074 ± 0.008	1.0000	1.193 ± 0.147	1.0000
62.5	5	0.660 ± 0.135	1.0000	0.116 ± 0.026	1.0000	1.196 ± 0.080	1.0000
125	5	0.620 ± 0.049	1.0000	0.151 ± 0.038	0.4031	1.201 ± 0.097	1.0000
250	5	0.490 ± 0.110	1.0000	0.106 ± 0.024	1.0000	1.319 ± 0.149	0.7538
500	5	0.710 ± 0.139	1.0000	0.206 ± 0.029	0.0269	1.336 ± 0.099	0.4233
Trend		p = 0.6294		p = 0.0031		p = 0.0671	

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

Table D-4. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Male and Female Mice in the Three-month Drinking Water Study of Sodium Metavanadate[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	2.840 ± 0.118		1.564 ± 0.011		1.546 ± 0.054	
31.3	5	3.050 ± 0.152	1.0000	1.527 ± 0.027	1.0000	1.399 ± 0.043	1.0000
62.5	5	2.608 ± 0.190	1.0000	1.523 ± 0.051	1.0000	1.588 ± 0.038	1.0000
125	5	2.910 ± 0.222	1.0000	1.557 ± 0.034	1.0000	1.851 ± 0.067	0.6122
250	5	2.480 ± 0.290	1.0000	1.471 ± 0.047	1.0000	2.130 ± 0.065	0.0660
500	5	2.430 ± 0.133	1.0000	1.426 ± 0.018	1.0000	2.699 ± 0.066	0.0032
Trend[d]		p = 0.9693		p = 0.9941		p < 0.001	
Female							
Exposure Concentration (mg/L)							
0	5	2.190 ± 0.196		0.969 ± 0.022		1.843 ± 0.092	
31.3	5	1.990 ± 0.163	1.0000	1.004 ± 0.010	0.5897	1.408 ± 0.167	1.0000
62.5	5	1.760 ± 0.130	1.0000	1.015 ± 0.023	0.2850	1.677 ± 0.215	1.0000
125	5	1.690 ± 0.207	1.0000	0.995 ± 0.018	1.0000	2.276 ± 0.177	1.0000
250	5	2.190 ± 0.051	1.0000	0.965 ± 0.032	1.0000	2.879 ± 0.080	0.2410
500	5	1.520 ± 0.177	1.0000	0.969 ± 0.026	1.0000	3.259 ± 0.073	0.0181
Trend		p = 0.9596		p = 0.6878		p < 0.001	

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

Table D-5. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Male and Female Rats in the Three-month Drinking Water Study of Vanadyl Sulfate[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	0.904 ± 0.179		0.345 ± 0.101		1.204 ± 0.142	
21.0	5	1.071 ± 0.202	1.0000	0.332 ± 0.126	1.0000	1.085 ± 0.046	1.0000
41.9	5	0.810 ± 0.168	1.0000	0.415 ± 0.099	1.0000	1.129 ± 0.066	1.0000
83.8	5	0.870 ± 0.155	1.0000	0.243 ± 0.083	1.0000	1.219 ± 0.021	1.0000
168	5	0.830 ± 0.151	1.0000	0.332 ± 0.065	1.0000	1.252 ± 0.088	1.0000
335	5	0.760 ± 0.051	1.0000	0.278 ± 0.041	1.0000	1.368 ± 0.026	0.4568
Trend[d]		p = 0.7827		p = 0.5932		p = 0.0069	
Female							
Exposure Concentration (mg/L)							
0	5	0.640 ± 0.051		0.222 ± 0.045		0.948 ± 0.129	
21.0	5	0.946 ± 0.096	0.1049	0.181 ± 0.038	1.0000	0.920 ± 0.053	1.0000
41.9	5	0.680 ± 0.106	1.0000	0.211 ± 0.057	1.0000	1.218 ± 0.060	0.1861
83.8	5	0.930 ± 0.041	0.0880	0.319 ± 0.075	1.0000	1.001 ± 0.076	1.0000
168	5	0.920 ± 0.142	0.2182	0.315 ± 0.102	1.0000	1.041 ± 0.039	1.0000
335	5	0.620 ± 0.150	1.0000	0.196 ± 0.039	1.0000	1.143 ± 0.039	0.7041
Trend		p = 0.3786		p = 0.3517		p = 0.0850	

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

Table D-6. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Male and Female Mice in the Three-month Drinking Water Study of Vanadyl Sulfate[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	3.120 ± 0.141		1.595 ± 0.037		1.503 ± 0.048	
21.0	5	2.850 ± 0.329	1.0000	1.671 ± 0.062	0.8758	1.675 ± 0.081	0.9798
41.9	5	2.360 ± 0.193	1.0000	1.576 ± 0.062	1.0000	1.537 ± 0.031	1.0000
83.8	5	2.620 ± 0.252	1.0000	1.487 ± 0.118	1.0000	1.767 ± 0.069	0.2030
168	5	2.380 ± 0.119	1.0000	1.580 ± 0.033	1.0000	1.775 ± 0.087	0.2030
335	5	2.440 ± 0.083	1.0000	1.660 ± 0.054	1.0000	2.024 ± 0.049	0.0016
Trend[d]		p = 0.9948		p = 0.4495		p < 0.001	
Female							
Exposure Concentration (mg/L)							
0	5	2.710 ± 0.277		1.265 ± 0.054		1.296 ± 0.135	
21.0	5	1.830 ± 0.062	1.0000	1.057 ± 0.041	1.0000	1.821 ± 0.147	0.3348
41.9	5	2.420 ± 0.292	1.0000	1.201 ± 0.092	1.0000	1.537 ± 0.180	1.0000
83.8	5	1.970 ± 0.176	1.0000	1.137 ± 0.049	1.0000	1.741 ± 0.199	0.4568
168	5	2.020 ± 0.175	1.0000	1.187 ± 0.034	1.0000	1.902 ± 0.120	0.1182
335	5	2.400 ± 0.373	1.0000	1.310 ± 0.082	1.0000	2.267 ± 0.327	0.0181
Trend		p = 0.6553		p = 0.1871		p = 0.0031	

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

Appendix E. Supplemental Data

Tables with supplemental data can be found here: https://doi.org/10.22427/NTP-DATA-TOX-106.

E.1. Perinatal and Three-month Sodium Metavanadate Study – Rats

E.1.1. Data Tables

I01 – Animal Removal Summary
C9404303_I01_Animal_Removal_Summary.pdf

I02 – Animal Removals
C9404303_I02_Animal_Removals.pdf

I03 – Growth Curve
C9404303_I03_Growth_Curve.pdf

I03C – Growth Curve
C9404303_I03C_Growth_Curve.pdf

I04 – Mean Body Weight Summary
C9404303_I04_Mean_Body_Weight_Summary.pdf

I04G – Mean Body Weight Gain
C9404303_I04G_Mean_Body_Weight_Gain.pdf

I05 – Clinical Observations Summary
C9404303_I05_Clinical_Observations_Summary.pdf

I05P – Pup Clinical Observations Summary
C9404303_I05P_Pup_Clinical_Observations_Summary.pdf

I07 – Mean Water Consumption
C9404303_I07_Mean_Water_Consumption.pdf

I08 – Mean Test Compound Consumption
C9404303_I08_Mean_Test_Compound_Consumption.pdf

PA02R – Neoplastic Lesion Summary with Percent and Litter Incidence
C9404303_PA02R_Neoplastic_Lesion_Summary_with_Percent_and_Litter_Incidence.pdf

PA03R – Non-Neoplastic Summary with Percent and Litter Incidence
C9404303_PA03R_Non-Neoplastic_Lesion_Summary_with_Percent_and_Litter_Incidence.pdf

PA05R – Incidence Rates of Neoplastic Lesions with Litter Incidence
C9404303_PA05R_Incidence_Rates_of_Neoplastic_Lesions_with_Litter_Incidence_Systemic_Lesions_Abridged.pdf

PA06 – Organ Weight Summary
C9404303_PA06_Organ_Weight_Summary.pdf

PA10R – Statistical Analysis of Nonneoplastic Lesions with Litter Incidence
C9404303_PA10R_Statistical_Analysis_of_Nonneoplastic_Lesions_with_Litter_Incidence.pdf

PA14 – Individual Animal Pathology Data
C9404303_PA14_Individual_Animal_Pathology_Data.pdf

PA18R – Non-Neoplastic Lesion Summary with Mean Severity Grade and Litter Incidence
C9404303_PA18R_Non-Neoplastic_Lesion_Summary_with_Mean_Severity_Grade_and_Litter_Incidence.pdf

PA41 – Clinical Chemistry Summary
C9404303_PA41_Clinical_Chemistry_Summary.pdf

PA43 – Hematology Summary
C9404303_PA43_Hematology_Summary.pdf

PA44 – Urinalysis Data Summary
C9404303_PA44_Urinalysis_Data_Summary.pdf

PA46 – Summary of Gross Pathology
C9404303_PA46_Summary_of_Gross_Pathology.pdf

PA48 – Summary of Tissue Concentration
C9404303_PA48_Summary_of_Tissue_Concentration.pdf

R01 – Multigeneration Cross-Reference
C9404303_R01_Multigeneration_Cross_Reference.pdf

R02 – Reproductive Performance Summary
C9404303_R02_Reproductive_Performance_Summary.pdf

R03 – Summary of Litter Data
C9404303_R03_Summary_of_Litter_Data.pdf

R06 – Andrology Summary
C9404303_R06_Andrology_Summary.pdf

R16 – Pubertal Markers Summary
C9404303_R16_Pubertal_Markers_Summary.pdf

R19 – Pup Mean Body Weight Summary
C9404303_R19_Pup_Mean_Body_Weight_Summary.pdf

R19C – Pup Growth Curve
C9404303_R19C_Pup_Growth_Curve.pdf

R19G – Pup Mean Body Weight Gain
C9404303_R19G_Pup_Mean_Body_Weight_Gain.pdf

Vaginal Cytology Markov Model
C9404303_Vaginal_Cytology_Markov_Model.pdf

Vaginal Cytology Plots
C9404303_Vaginal_Cytology_Plots.pdf

Vaginal Cytology Summary
C9404303_Vaginal_Cytology_Summary.pdf

E.1.2. Individual Animal Data

Individual Animal Andrology Data
C9404303_Individual_Animal_Andrology_Data.xlsx

Individual Animal Body Weight Data
C9404303_Individual_Animal_Body_Weight_Data.xlsx

Individual Animal Clinical Chemistry Data
C9404303_Individual_Animal_Clinical_Chemistry_Data.xlsx

Individual Animal Clinical Observations Data
C9404303_Individual_Animal_Clinical_Observations_Data.xlsx

Individual Animal Consumption Data
C9404303_Individual_Animal_Consumption_Data.xlsx

Individual Animal Developmental Markers Data
C9404303_Individual_Animal_Developmental_Markers_Data.xlsx

Individual Animal Gross Pathology Data
C9404303_Individual_Animal_Gross_Pathology_Data.xlsx

Individual Animal Hematology Data
C9404303_Individual_Animal_Hematology_Data.xlsx

Individual Animal Histopathology Data
C9404303_Individual_Animal_Histopathology_Data.xlsx

Individual Animal Litter Data
C9404303_Individual_Animal_Litter_Data.xlsx

Individual Animal Organ Weight Data
C9404303_Individual_Animal_Organ_Weight_Data.xlsx

Individual Animal Pup Body Weight Data
C9404303_Individual_Animal_Pup_Body_Weight_Data.xlsx

Individual Pup Clinical Observations Data
C9404303_Individual_Animal_Pup_Clinical_Observations_Data.xlsx

Individual Animal Removal Reasons Data
C9404303_Individual_Animal_Removal_Reasons_Data.xlsx

Individual Animal Reproductive Performance Data
C9404303_Individual_Animal_Reproductive_Performance_Data.xlsx

Individual Animal Tissue Concentration Data
C9404303_Individual_Animal_Tissue_Concentration_Data.xlsx

Individual Animal Urinalysis Data
C9404303_Individual_Animal_Urinalysis_Data.xlsx

Individual Animal Whole Litter Weight Data
C9404303_Individual_Animal_Whole_Litter_Weight_Data.xlsx

E.2. Three-month Sodium Metavanadate Study – Mice

E.2.1. Data Tables

I01 – Animal Removal Summary
C9404304_I01_Animal_Removal_Summary.pdf

I02 – Animal Removals
C9404304_I02_Animal_Removals.pdf

I03 – Growth Curve
C9404304_I03_Growth_Curve.pdf

I03C – Growth Curve
C9404304_I03C_Growth_Curve.pdf

I04 – Mean Body Weight Summary
C9404304_I04_Mean_Body_Weight_Summary.pdf

I04G – Mean Body Weight Gain
C9404304_I04G_Mean_Body_Weight_Gain.pdf

I05 – Clinical Observations Summary
C9404304_I05_Clinical_Observations_Summary.pdf

I07 – Mean Water Consumption
C9404304_I07_Mean_Water_Consumption.pdf

I08 – Mean Test Compound Consumption
C9404304_I08_Mean_Test_Compound_Consumption.pdf

PA02 – Neoplastic Lesion Summary with Percent Incidence
C9404304_PA02_Neoplastic_Lesion_Summary_with_Percent_Incidence.pdf

PA03 – Non-neoplastic Lesion Summary with Percent Incidence
C9404304_PA03_Non-Neoplastic_Lesion_Summary_with_Percent_Incidence.pdf

PA05 – Incidence Rates of Neoplastic Lesions with Systemic Lesions Abridged
C9404304_PA05_Incidence_Rates_of_Neoplastic_Lesions_with_Systemic_Lesions_Abridged.pdf

PA06 – Organ Weights Summary
C9404304_PA06_Organ_Weights_Summary.pdf

PA08 – Statistical Analysis of Neoplastic Lesions
C9404304_PA08_Statistical_Analysis_of_Neoplastic_Lesions.pdf

PA10 – Statistical Analysis of Nonneoplastic Lesions
C9404304_PA10_Statistical_Analysis_of_Nonneoplastic_Lesions.pdf

PA14 – Individual Animal Pathology Data
C9404304_PA14_Individual_Animal_Pathology_Data.pdf

PA18 – Incidence Rates of Non-neoplastic Lesions by Anatomic Site with Average Severity Grade
C9404304_PA18_Incidence_C9404304_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grade.pdf

PA43 – Hematology Summary
C9404304_PA43_Hematology_Summary.pdf

PA46 – Summary of Gross Pathology
C9404304_PA46_Summay_of_Goss_Pathology.pdf

R06 – Andrology Summary
C9404304_R06_Andrology_Summary.pdf

Vaginal Cytology Markov Model
C9404304_Vaginal_Cytology_Markov_Model.pdf

Vaginal Cytology Plots
C9404304_Vaginal_Cytology_Plots.pdf

Vaginal Cytology Summary
C9404304_Vaginal_Cytology_Summary.pdf

E.2.2. Individual Animal Data

Individual Animal Andrology Data
C9404304_Individual_Animal_Andrology_Data.xlsx

Individual Animal Body Weight Data
C9404304_Individual_Animal_Body_Weight_Data.xlsx

Individual Animal Clinical Observations Data
C9404304_Individual_Animal_Clinical_Observations_Data.xlsx

Individual Animal Consumption Data
C9404304_Individual_Animal_Consumption_Data.xlsx

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Individual Animal Hematology Data
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Individual Animal Organ Weight Data
C9404304_Individual_Animal_Organ_Weight_Data.xlsx

Individual Animal Removal Reasons Data
C9404304_Individual_Animal_Removal_Reasons_Data.xlsx

E.3. Perinatal and Three-month Vanadyl Sulfate Study – Rats

E.3.1. Data Tables

I01 – Animal Removal Summary
C0800403_I01_Animal_Removal_Summary.pdf

I02 – Animal Removals
C0800403_I02_Animal_Removals.pdf

I03 – Growth Curve
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R02 – Reproductive Performance Summary
C0800403_R02_Reproductive_Performance_Summary.pdf

R03 – Summary of Litter Data
C0800403_R03_Summary_of_Litter_Data.pdf

R06 – Andrology Summary
C0800403_R06_Andrology_Summary.pdf

R16 – Pubertal Markers Summary
C0800403_R16_Pubertal_Markers_Summary.pdf

R19 – Pup Mean Body Weight Summary
C0800403_R19_Pup_Mean_Body_Weight_Summary.pdf

R19C – Pup Growth Curve
C0800403_R19C_Pup_Growth_Curve.pdf

R19G – Pup Mean Body Weight Gain
C0800403_R19G_Pup_Mean_Body_Weight_Gain.pdf

Vaginal Cytology Markov Model
C0800403_Vaginal_Cytology_Markov_Model.pdf

Vaginal Cytology Plots
C0800403_Vaginal_Cytology_Plots.pdf

Vaginal Cytology Summary
C0800403_Vaginal_Cytology_Summary.pdf

E.3.2. Individual Animal Data

Individual Animal Andrology Data
C0800403_Individual_Animal_Andrology_Data.xlsx

Individual Animal Body Weight Data
C0800403_Individual_Animal_Body_Weight_Data.xlsx

Individual Animal Clinical Chemistry Data
C0800403_Individual_Animal_Clinical_Chemistry_Data.xlsx

Individual Animal Clinical Observations Data
C0800403_Individual_Animal_Clinical_Observations_Data.xlsx

Individual Animal Consumption Data
C0800403_Individual_Animal_Consumption_Data.xlsx

Individual Animal Developmental Markers Data
C0800403_Individual_Animal_Developmental_Markers_Data.xlsx

Individual Animal Gross Pathology Data
C0800403_Individual_Animal_Gross_Pathology_Data.xlsx

Individual Animal Hematology Data
C0800403_Individual_Animal_Hematology_Data.xlsx

Individual Animal Histopathology Data
C0800403_Individual_Animal_Histopathology_Data.xlsx

Individual Animal Litter Data
C0800403_Individual_Animal_Litter_Data.xlsx

Individual Animal Organ Weight Data
C0800403_Individual_Animal_Organ_Weight_Data.xlsx

Individual Animal Pup Body Weight Data
C0800403_Individual_Animal_Pup_Body_Weight_Data.xlsx

Individual Animal Pup Clinical Observations Data
C0800403_Individual_Animal_Pup_Clinical_Observations_Data.xlsx

Individual Animal Removal Reasons Data
C0800403_Individual_Animal_Removal_Reasons_Data.xlsx

Individual Animal Reproductive Performance Data
C0800403_Individual_Animal_Reproductive_Performance_Data.xlsx

Individual Animal Tissue Concentration Data
C0800403_Individual_Animal_Tissue_Concentration_Data.xlsx

Individual Animal Urinalysis Data
C0800403_Individual_Animal_Urinalysis_Data.xlsx

Individual Whole Litter Weight Data
C0800403_Individual_Animal_Whole_Litter_Weight_Data.xlsx

E.4. Three-month Vanadyl Sulfate Study – Mice

E.4.1. Data Tables

I01 – Animal Removal Summary
C0800404_I01_Animal_Removal_Summary.pdf

I02 – Animal Removals
C0800404_I02_Animal_Removals.pdf

I03 – Growth Curve
C0800404_I03_Growth_Curve.pdf

I03C – Growth Curve
C0800404_I03C_Growth_Curve.pdf

I04 – Mean Body Weight Summary
C0800404_I04_Mean_Body_Weight_Summary.pdf

I04G – Mean Body Weight Gain
C0800404_I04G_Mean_Body_Weight_Gain.pdf

I05 – Clinical Observations Summary
C0800404_I05_Clinical_Observations_Summary.pdf

I07 – Mean Water Consumption
C0800404_I07_Mean_Water_Consumption.pdf

I08 – Mean Test Compound Consumption
C0800404_I08_Mean_Test_Compound_Consumption.pdf

PA02 – Neoplastic Lesion Summary with Percent Incidence
C0800404_PA02_Neoplastic_Lesion_Summary_with_Percent_Incidence.pdf

PA03 – Non-Neoplastic Lesion Summary with Percent Incidence
C0800404_PA03_Non-Neoplastic_Lesion_Summary_with_Percent_Incidence.pdf

PA05 – Incidence Rates of Neoplastic Lesions with Systemic Lesions Abridged
C0800404_PA05_Incidence_Rates_of_Neoplastic_Lesions_with_Systemic_Lesions_Abridged.pdf

PA06 – Organ Weights Summary
C0800404_PA06_Organ_Weights_Summary.pdf

PA08 – Statistical Analysis of Neoplastic Lesions
C0800404_PA08_Statistical_Analysis_of_Neoplastic_Lesions.pdf

PA10 – Statistical Analysis of Nonneoplastic Lesions
C0800404_PA10_Statistical_Analysis_of_Nonneoplastic_Lesions.pdf

PA14 – Individual Animal Pathology Data
C0800404_PA14_Individual_Animal_Pathology_Data.pdf

PA18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grade
C0800404_PA18_Incidence_Rates_of_Non-Neoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grade.pdf

PA43 – Hematology Summary
C0800404_PA43_Hematology_Summary.pdf

PA46 – Summary of Gross Pathology
C0800404_PA46_Summary_of_Gross_Pathology.pdf

R06 – Andrology Summary
C0800404_R06_Andrology_Summary.pdf

Vaginal Cytology Markov Model
C0800404_Vaginal_Cytology_Markov_Model.pdf

Vaginal Cytology Plots
C0800404_Vaginal_Cytology_Plots.pdf

Vaginal Cytology Summary
C0800404_Vaginal_CytologySummary.pdf

E.4.2. Individual Animal Data

Individual Animal Andrology Data
C0800404_Individual_Animal_Andrology_Data.xlsx

Individual Animal Body Weight Data
C0800404_Individual_Animal_Body_Weight_Data.xlsx

Individual Animal Clinical Observations Data
C0800404_Individual_Animal_Clinical_Observations_Data.xlsx

Individual Animal Consumption Data
C0800404_Individual_Animal_Consumption_Data.xlsx

Individual Animal Gross Pathology Data
C0800404_Individual_Animal_Gross_Pathology_Data.xlsx

Individual Animal Hematology Data
C0800404_Individual_Animal_Hematology_Data.xlsx

Individual Animal Histopathology Data
C0800404_Individual_Animal_Histopathology_Data.xlsx

Individual Animal Organ Weight Data
C0800404_Individual_Animal_Organ_Weight_Data.xlsx

Individual Animal Removal Reasons Data
C0800404_Individual_Animal_Removal_Reasons_Data.xlsx

E.5. Genetic Toxicology

E.5.1. Sodium Metavanadate (13718-26-8) in Micronucleus Study in Sprague Dawley Rats

G04 – In Vivo Micronucleus Summary
G94043B_G04_In_Vivo_Micronucleus_Summary_Data.pdf

Individual Animal In Vivo Micronucleus Data
G94043B_Individual_Animal_In_Vivo_Micronucleus_Data.xlsx

E.5.2. Sodium Metavanadate (13718-26-8) in Micronucleus Study in B6C3F1/N Mice

G04 – In Vivo Micronucleus Summary
G94043C_G04_In_Vivo_Micronucleus_Summary_Data.pdf

Individual Animal In Vivo Micronucleus Data
G94043C_Individual_Animal_In_Vivo_Micronucleus_Data.xlsx

E.5.3. Sodium Metavanadate (13718-26-8) in *Salmonella/E.coli* Mutagenicity Test or Ames Test

G06 – Ames Summary Data
G94043_G06_Ames_Summary_Data.pdf

E.5.4. Vanadyl Sulfate (27774-13-6) in Micronucleus Study in Sprague Dawley Rats

G04 – In Vivo Micronucleus Summary
G08004B_G04_In_Vivo_Micronucleus_Summary_Data.pdf

Individual Animal In Vivo Micronucleus Data
G08004B_Individual_Animal_In_Vivo_Micronucleus_Data.xlsx

E.5.5. Vanadyl Sulfate (27774-13-6) in Micronucleus Study in B6C3F1/N Mice

G04 – In Vivo Micronucleus Summary
G08004C_G04_In_Vivo_Micronucleus_Summary_Data.pdf

Individual Animal In Vivo Micronucleus Data
G08004C_Individual_Animal_In_Vivo_Micronucleus_Data.xlsx

E.5.6. Vanadyl Sulfate (27774-13-6) in *Salmonella/E.coli* Mutagenicity Test or Ames Test

G06 – Ames Summary Data
G08004_G06_Ames_Summary_Data.pdf

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