3-Month Toxicity Studies of Tetravalent and Pentavalent Vanadium Compounds in Hsd:Sprague Dawley SD Rats and B6C3F1/N Mice via Drinking Water Exposure

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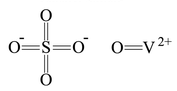
Background

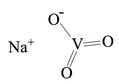
- Vanadium is a naturally occurring metal and exists in various oxidation states from -1 to +5
  - Vanadium +3 to +5 species are considered the most stable, however, oxidation-reduction in the environment and following ingestion are likely
- Natural processes (e.g., volcanic eruptions) and anthropogenic sources (e.g., fly ash from coal fired power plants) release vanadium to the environment
  - Industrial waste containing vanadium is also imported into the United States for use in steel production
- Exposure to vanadium may occur via ingestion
  - Vanadyl sulfate is sold as a dietary supplement for diabetes and high cholesterol
  - Sodium metavanadate was selected for study as a representative pentavalent species, a potential drinking water contaminant
- There is uncertainty regarding the human health implications for long term exposure to vanadium ingestion
- NTP performed 14-day toxicity studies of tetravalent (+4; vanadyl sulfate) and pentavalent (+5; sodium metavanadate) compounds in rats and mice (Figure 1)

Figure 1. Chemical Structures of the Soluble Vanadium Compounds Tested

Vanadyl Sulfate



Sodium Metavanadate

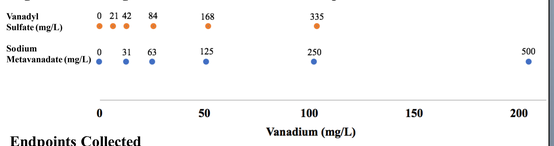


Methods

Animal Model and Exposure

- Time-mated F0 Hsd:Sprague Dawley SD rats (n=16/group) were exposed to vanadyl sulfate or sodium metavanadate via drinking water beginning on GD6
  - Groups of 15 male and 15 female F1 animals were exposed during gestation, lactation and 13-weeks post-weaning (5 animals for biological sampling)
- Groups of 10 male and 10 female B6C3F1/N mice were exposed to vanadyl sulfate or sodium metavanadate for 13 weeks via drinking water
- Drinking water concentrations were selected based on 14-day data; concentrations for vanadyl sulfate (31% vanadium) ranged from 21-335 mg/L, and from 31-500 mg/L for sodium metavanadate (41% vanadium)
  - Figure 2 illustrates the overlap in *total vanadium* concentration (mg/L) between the compounds

Figure 2. Drinking Water Concentrations of Compounds and Vanadium



Endpoints Collected

- Rats
  - Gestational, lactational F0 body weights, F1 body weights
  - Fertility, fecundity, pup survival
  - Water consumption
  - Clinical pathology, organ weights, histopathology (still under evaluation)
  - Measurement of vanadium concentration in plasma and urine
    - At the end of the 13-week post-weaning exposure period, 5 rats per sex per group, for both compounds, were placed in metabolism cages (24 hours) for collection of urine
    - After 24 hours, rats were euthanized and blood was collected
- Mice
  - Survival, body weights, water consumption
  - Hematology, organ weights, histopathology (still under evaluation)

Analysis of Urine and Plasma for Total Vanadium

- Speciation of vanadium in biological fluids was not possible, thus total vanadium was measured
- Samples were thawed, processed by acid digestion with heat, spiked with internal standard (praseodymium) and diluted with DI H_2O for analysis
- Samples were analyzed using inductively-coupled plasma-mass spectrometry on a Thermo X-Series, ThermoFisher Scientific (Waltham, MA)

Table 1. Method Validation Summary[1]

Parameter	Outcome
Limit of Detection	0.155 ng/mL
Lower Limit of Quantitation	5.00 ng/mL
Concentration range (8-point curve)	2.5 – 2,500 ng/mL
	Accuracy[2]: -1.4 to 5.1%
Intra-day Evaluation	Accuracy: 8.0-17.3%
	Precision[3]: ≤ 1.8%
Inter-day Evaluation	Accuracy: 3.0-17.3%
	Precision: ≤ 3.1%
Stability[4]: Ambient Autosampler Extract Storage, Refrigerator Extract Storage, and Freeze-Thaw	97.7 - 126%,
Short and Long-Term Storage Stability	100 - 122% of Day 0
	Precision: ≤ 2.6%

[1] Data presented are for plasma only; a similar validation was conducted for urine (data not shown)
[2] Accuracy was determined as relative error
[3] Precision was determined as relative standard deviation
[4] Stability presented as percent of day 0

Results for Hsd:Sprague Dawley SD Rats

Disclaimer: Information shown below have not been subjected to the NTP data review processes (e.g. evaluation for outliers, litter-based calculations). Some data from the studies, including histopathology are still under evaluation.

Vanadyl Sulfate

Survival

- There was no impact on F0 dam survival during gestation or lactation or F1 survival during lactation or post-weaning

Table 2. F1 Litter Data for Vanadyl Sulfate Exposed Rats[1]

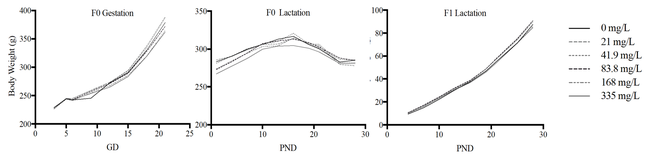
	0 mg/L	21 mg/L	41.9 mg/L	83.8 mg/L	168 mg/L	335 mg/L
Live Pups PND 0	14.2 ± 0.4 (15)	13.9 ± 0.5 (16)	13.1 ± 0.8 (16)	11.8 ± 0.8 (16)	13.3 ± 0.8 (15)	12.8 ± 0.7 (15)
% Live Birth	98.24 ± 0.78 (15)	96.81 ± 1.29 (16)	94.97 ± 2.11 (16)	91.64 ± 4.27 (16)	96.52 ± 2.09 (15)	99.11 ± 0.89 (15)
Live Pups PND 4[2]	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)

[1] Data represent averages ± SEM (N, litters)
[2] Average live pups on PND 4 before litters were standardized

Body weights

- There were no impacts on body weight during gestation or lactation for F0 females and no impact on F1 body weights during lactation or post-weaning (data not shown)
- Terminal F1 body weights were within 5% of controls

Figure 3. F0 Body Weights During Gestation and F0 and F1 Body Weights During Lactation



Water and chemical consumption

- There was lower water consumption in the 168 and 335 mg/L groups during gestation and lactation (335 mg/L only); all others were within 10% of controls
- Post-weaning, there was lower water consumption for F1 males ≥ 83.8 mg/L and females in the 335 mg/L group
- Due to lower water consumption, chemical consumption in the higher groups was slightly less than dose-proportional

Table 3. Water and Vanadyl Sulfate Consumption During Gestation, Lactation and Post-weaning

Study Phase	Measurement	0 mg/L	21 mg/L	41.9 mg/L	83.8 mg/L	168 mg/L	335 mg/L
Gestation (GD6-21)	Water (g/day)	42.1 ± 1.9	39.8 ± 0.9	41.1 ± 1.4	37.0 ± 1.5	31.4 ± 1.2	26.9 ± 0.9
	Chemical (mg/kg/day)	---	2.8 ± 0.1	5.8 ± 0.4	10.6 ± 0.4	18.3 ± 0.5	31.3 ± 0.9
Lactation (PND 1-13)	Water (g/day)	62.1 ± 1.9	61.9 ± 1.0	63.0 ± 1.9	61.6 ± 1.8	57.4 ± 1.8	54.1 ±1.4
	Chemical (mg/kg/day)	---	4.4 ± 0.1	8.9 ± 0.2	17.5 ± 0.4	33.0 ± 0.9	62.8 ± 1.4
F1 Males (PND 28-119)	Water (g/day)	29.5 ± 1.3	26.2 ± 0.8	27.5 ± 0.8	25.4 ± 0.8	24.5 ± 0.6	21.6 ± 0.4
	Chemical (mg/kg/day)	---	1.8 ± 0.1	3.7 ± 0.1	6.9 ± 0.2	13.7 ± 0.3	25.1 ± 0.4
F1 Females (PND 28-119)	Water (g/day)	21.6 ± 1.1	19.9 ± 0.3	22.4 ± 0.9	20.4 ± 0.7	21.0 ± 0.7	17.6 ± 0.4
	Chemical (mg/kg/day)	---	2.1 ± 0.1	4.4 ± 0.1	8.2 ± 0.3	16.6 ± 0.4	29.8 ± 0.6

Sodium Metavanadate

Survival

- There was no impact on survival during gestation
- Moribundity resulted in removal of F0 animals during parturition and throughout lactation in the 250 and 500 mg/L groups
- Lower pup survival was observed in 500 mg/L pups from PND 1-10; survival after PND 10 was similar between exposed groups and controls

Table 4. F1 Litter Data for Sodium Metavanadate Exposed Rats[1]

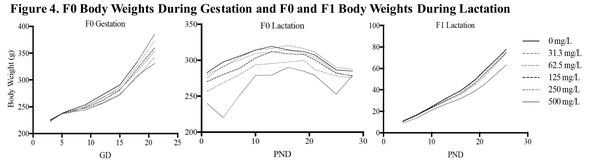
	0 mg/L	31.3 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L
Live Pups PND 0	12.3 ± 0.5 (16)	10.9 ± 1.1 (14)	12.4 ± 1.0 (16)	12.1 ± 1.2 (16)	10.5 ± 1.2 (13)	7.6 ± 1.2 (15)
% Live Birth	95.09 ± 3.11 (16)	97.99 ± 1.43 (14)	98.66 ± 0.97 (16)	91.36 ± 6.19 (16)	98.05 ± 1.35 (12)	73.06 ± 9.64 (15)
Live Pups PND 4[2]	11.8 ± 0.9 (16)	10.2 ± 1.2 (14)	12.1 ± 1.1 (16)	13.4 ± 0.6 (14)	11.1 ± 1.0 (11)	6.3 ± 1.5 (12)
Survival PND 1-4 (%)	93.75 ± 6.25 (16)	92.06 ± 7.13 (14)	93.75 ± 6.25 (16)	91.77 ± 6.61 (15)	98.59 ± 0.95 (11)	68.75 ± 13.46 (12)
Survival PND 4-7 (%)	100 ± 0.0 (15)	100 ±0.0 (12)	100 ± 0.0 (14)	99.11 ± 0.89 (14)	100 ± 0.0 (11)	81.25± 12.38 (8)
Survival PND 7-10 (%)	100 ± 0.0 (15)	100 ± 0.0 (12)	99.11 ± 0.89 (14)	100 ± 0.0 (14)	100 ± 0.0 (11)	85.71 ± 14.29 (7)

[1] Data represent averages ± SEM (N, litters)
[2] Average live pups on PND 4 before litters were standardized

Body weights

- There were lower body weights in dams during gestation and lactation that was proportional to sodium metavanadate exposure
- Body weight effects were only seen in pups at 500 mg/L
- At the end of the study for F1 pups (13-weeks post weaning), lower body weights were seen in males at ≥ 125 mg/L (-10 to -20%) and in females at 500 mg/L (-12%)

Figure 4. F0 Body Weights During Gestation and F0 and F1 Body Weights During Lactation



Water and chemical consumption

- There was lower water consumption in the 250 and 500 mg/L groups during gestation, lactation and post-weaning for male and female F1 offspring
- Due to lower water consumption, chemical consumption in the higher groups was slightly less than dose-proportional

Table 5. Water and Vanadyl Sulfate Consumption During Gestation, Lactation and Post-weaning

Study Phase	Measurement	0 mg/L	31.3 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L
Gestation (GD6-21)	Water (g/day)	39.8 ± 1.0	37.8 ± 1.6	38.4 ± 1.3	33.5 ± 1.3	27.8 ± 1.4	23.5 ± 0.7
	Chemical (mg/kg/day)	---	4.2 ± 0.2	8.3 ± 0.2	14.7 ± 0.5	25.0 ± 1.2	42.74 ± 1.1
Lactation (PND 1-13)	Water (g/day)	64.1 ± 3.0	64.3 ± 1.1	66.7 ± 1.1	62.7 ± 1.1	55.9 ± 2.8	53.5 ± 4.0
	Chemical (mg/kg/day)	---	6.7 ± 0.1	14.0 ± 0.3	27.0 ± 0.6	50.0 ± 2.7	99.5 ± 7.7
F1 Males (PND 28-119)	Water (g/day)	24.3 ± 1.3	28.5 ± 0.5	28.6 ± 0.5	25.3 ± 0.7	21.5 ± 0.4	15.8 ± 0.9
	Chemical (mg/kg/day)	---	2.7 ± 0.1	5.4 ± 0.1	10.3 ± 0.4	18.0 ± 0.4	35.9 ± 1.4
F1 Females (PND 28-119)	Water (g/day)	21.3 ± 0.2	21.3 ± 1.0	21.6 ± 0.5	20.3 ± 0.7	15.6 ± 0.6	12.6 ± 0.5
	Chemical (mg/kg/day)	---	3.0 ± 0.1	6.4 ± 0.1	12.3 ± 0.2	18.7 ± 0.7	40.2 ± 4.5

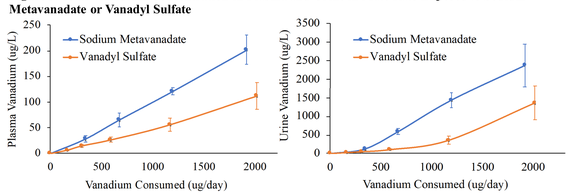
Analysis for Total Vanadium in Plasma and Urine

Table 6. Plasma and Urine Vanadium (V) Concentration in Rats Exposed to Vanadyl Sulfate

	Drinking Water Concentrations		Consumption		Vanadium Concentration (ug/L)[4]	
	Vanadyl Sulfate (mg/L)	V (mg/L)[1]	Water (g/day)[2]	V (μg/day)[3]	Plasma	Urine
Male	0	0	27.5	0.0	0.0 ± 0.0	0.0 ± 0.0
	21.0	6.5	25.3	164.3	6.6 ± 1.7	26.0 ± 8.2
	41.9	13.0	23.6	306.8	14.9 ± 3.2	50.3 ± 12.9
	83.8	26.0	22.7	589.2	26.5 ± 4.8	104.6 ± 16.5
	167.5	51.9	22.5	1167.8	56.0 ± 13.1	355.0 ± 107.1
	335.0	103.9	19.4	2018.8	112.3 ± 26.1	1364.0 ± 446
Female	0	0	21.7	0.0	0.7 ± 0.3	2.4 ± 1.6
	21.0	6.5	19.2	124.4	5.6 ± 0.7	17.0 ± 5.5
	41.9	13.0	23.2	300.8	16.6 ± 5.3	37.9 ± 17.1
	83.8	26.0	18.8	487.1	37.6 ± 4.9	71.8 ± 24.3
	167.5	51.9	19.1	993.3	50.3 ± 8.0	149.3 ± 23.8
	335.0	103.9	15.9	1654.3	139.6 ± 16.1	613.4 ± 58.4

[1] V (mg/L) calculated as 31% of vanadyl sulfate by molecular weight
[2] Final week of the study (PND 112-119)
[3] V consumed calculated as [water consumed (g/day)*V concentration (mg/L)]; assumes drinking water density of 1g/mL
[4] Data presented are average (n=3-5) ± standard deviation

Figure 5. Total Vanadium Concentration in Plasma and Urine in Male Rats Exposed to Sodium Metavanadate or Vanadyl Sulfate



Based on the calculated amount of vanadium consumed and the level of of vanadium in plasma and urine (Tables 6 and 7), there was higher absorption and exposure for animals exposed to sodium metavanadate compared to vanadyl sulfate (Figure 5), when consuming a similar amount of vanadium; female rats showed a similar pattern

Table 7. Plasma and Urine Vanadium (V) Concentration in Rats Exposed to Sodium Metavanadate

	Drinking Water Concentrations		Consumption		Vanadium Concentration (ug/L)[5]	
	Sodium Metavanadate (mg/L)[1]	V (mg/L)[2]	Water (g/day)[3]	V (μg/g/day)[4]	Plasma	Urine
Male	0	0	25.7	0.0	0.0 ± 0.0	0.0 ± 0.0
	31.3	12.8	26.7	342.2	28.4 ± 6.2	120.0 ± 32.2
	62.5	25.6	26.2	670.6	65.4 ± 13.3	588.2 ± 75.6
	125.0	51.3	23.3	1194.1	121.3 ± 7.0	1437.9 ± 195.8
	250.0	102.5	18.7	1917.8	202.1 ± 28.1	2376.0 ± 575.6
Female	0	0	20.7	0.0	0.0 ± 0.0	0.0 ± 0.0
	31.3	12.8	21.2	272.0	27.8 ± 6.7	65.1 ± 40.9
	62.5	25.6	20.2	517.9	72.2 ± 20.5	214.9 ± 71.8
	125.0	51.3	19.9	1017.8	165.5 ± 37.7	1026.6 ± 580.0
	250.0	102.5	14.9	1524.2	297.9 ± 24.8	1588.0 ± 140.1

[1] Pup toxicity during lactation resulted in insufficient animals in the 500 mg/L group to populate the biological sampling cohort
[2] V (mg/L) calculated as 41% of sodium metavanadate by molecular weight
[3] Final week of the study (PND 112-119)
[4] V consumed calculated as [water consumed (g/day)*V concentration (mg/L)]; assumes drinking water density of 1g/mL
[5] Data presented are average (n=3-5) ± standard deviation

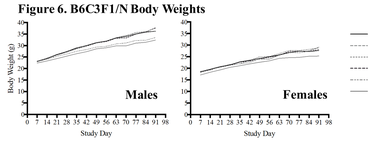
Results for B6C3F1/N Mice

Vanadyl Sulfate

Survival and Body Weights

- There was no impact on survival and terminal body weights were within 11% of controls

Figure 6. B6C3F1/N Body Weights



Water and chemical consumption

- There was a slight decrease in water consumption at 335 mg/L in males; no change in females

Table 8. Water and Vanadyl Sulfate Consumption During the Final Study Week

Measurement	0 mg/L	21 mg/L	41.9 mg/L	83.8 mg/L	168 mg/L	335 mg/L
Males						
Water (g/day)	3.5 ± 0.1	3.3 ± 0.1	3.5 ± 0.1	3.5 ± 0.1	3.5 ± 0.1	3.1 ± 0.1
Chemical (mg/kg/day)	---	1.9 ± 0.1	4.1 ± 0.2	8.2 ± 0.3	18.1 ± 0.6	33.3 ± 1.1
Females						
Water (g/day)	2.6 ± 0.0	2.5 ± 0.1	2.4 ± 0.0	2.5 ± 0.0	2.4 ± 0.0	2.4 ± 0.0
Chemical (mg/kg/day)	---	1.9 ± 0.1	3.5 ± 0.1	7.7 ± 0.3	14.6 ± 0.6	31.8 ± 0.7

Organ weights and hematology

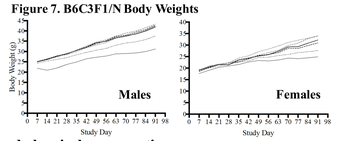
- There were no changes in organ weights or hematology considered related to vanadyl sulfate exposure

Sodium Metavanadate

Survival and Body Weights

- One male mouse in the 500 mg/L group was removed from study in week 3, all other animals survived to study termination
- On day 91, male body weights were -26% vs. controls in the 500 mg/L group, all others were within 11% of controls; females were -23% and -14% vs. controls for 500 and 250 mg/L, respectively

Figure 7. B6C3F1/N Body Weights



Water and chemical consumption

- There was a decrease in water consumption at 500 mg/L in males and females, resulting in less than dose proportional intake of sodium metavanadate

Table 9. Water and Sodium Metavanadate Consumption During the Final Study Week

Measurement	0 mg/L	31.3 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L
Males						
Water (g/day)	3.5 ± 0.1	3.4 ± 0.1	3.3 ± 0.1	3.3 ± 0.1	3.2 ± 0.1	2.5 ± 0.1
Chemical (mg/kg/day)	---	2.5 ± 0.1	5.1 ± 0.2	9.9 ± 0.5	22.0 ± 0.7	40.6 ± 1.3
Females						
Water (g/day)	3.0 ± 0.1	2.8 ± 0.1	2.6 ± 0.0	2.8 ± 0.0	2.8 ± 0.0	2.1 ± 0.1
Chemical (mg/kg/day)	---	2.6 ± 0.1	4.9 ± 0.2	11.6 ± 0.3	25.3 ± 0.8	42.7 ± 1.1

Organ weights and hematology

- Thymus weights were lower in sodium metavanadate-exposed mice; 250 mg/L (male) and 500 mg/L
- There were increases in erythrocytes (17-25% at 500 mg/L) and reticulocytes (38-50% at 500 mg/L) and small deceases in hematocrit and hemoglobin in males and females exposed to sodium metavanadate

Conclusions

- Vanadyl sulfate, up to 335 mg/L in drinking water, was well tolerated in adult mice and time-mated rats during gestation and lactation, and their pups during lactation and up to 13 weeks post-weaning
- Dams/pups exposed to sodium metavanadate at 250 and 500 mg/L in drinking water exhibited moribundity at birth, failure to thrive, and higher moribundity during lactation
- Lower body weights were observed in dams during gestation and lactation, and in pups continuing until study termination, 13 weeks post-weaning
- Plasma and urine collected from rats exposed to both compounds were evaluated for total vanadium
  - Based on calculation of vanadium consumption, the amount of vanadium absorbed and present in plasma and urine is higher for animals exposed to sodium metavanadate compared to vanadyl sulfate
  - Levels of vanadium in males and females were similar when taking into account compound and vanadium consumption
- Sodium metavanadate in drinking water resulted in lower body weights and water consumption at 500 mg/L in adult B6C3F1/N mice
  - Mice exposed to higher concentrations of sodium metavanadate also had lower thymus weights
  - Mice exposed to higher (125, 150, 500 mg/L) concentrations of sodium metavanadate exhibited increases in erythrocytes and reticulocytes and deceases in hematocrit and hemoglobin

Acknowledgments

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