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

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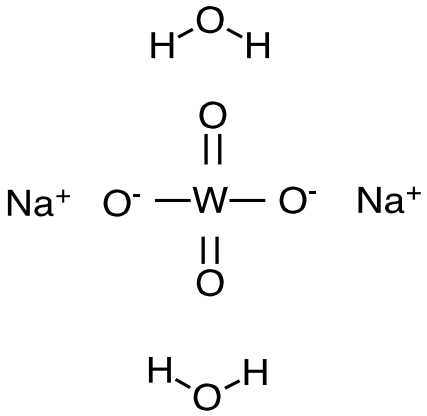
Background

- Tungsten is present naturally in the environment and can enter waterways through weathering of rocks and soil

- Hence, tungsten was nominated for study due to concerns about potential widespread human exposure via contaminated drinking water

- Sodium Tungstate dihydrate (ST) was selected for study because it is a naturally occurring form of tungsten and most water soluble

- Drinking water was selected as the most likely route of exposure for the general population



Subchronic Studies

Subchronic Studies: Design

	Rats	Mice
Duration	GD6-PND21 + 13 weeks	13 weeks
Concentration	0, 125, 250, 500, 1000, 2000 mg/L	0, 125, 250, 500, 1000, 2000 mg/L
Number of animals (per sex per concentration)	10	10
Endpoints	Survival Body Weights Water Consumption Clinical Observations Hematology Clinical Chemistry Urinalysis Organ Weights Histopathology Tungsten conc. in blood and urine Genotoxicity – micronucleus and comet assay	Survival Body Weights Water Consumption Clinical Observations Hematology Organ Weights Histopathology Tungsten conc. in blood and urine Genotoxicity – micronucleus and comet assay

Results: Subchronic Rat Studies

- Decrease in water consumption for 1,000 and 2,000 mg/L males and females; overall reductions of 27% and 42% for males and females, respectively, in the 2,000 mg/L groups compared to vehicle controls

- No exposure-related effects on pregnancy status, maternal survival, or the number of dams that littered *at any of the exposure concentrations tested*

- Significant decrease in mean body weights of dams in 1000 and 2000 mg/L (~10% and 18%, respectively) at the end of lactation

- Mean body weights of pups (male and female combined) on postnatal day 21 in 2000 mg/L significantly decreased by ~14%

Results: Subchronic Rat Studies Contd.

- Serum insulin concentrations were significantly decreased in the 2000 mg/L males; serum glucose unchanged
- Kidney- major target organ of toxicity
  - Renal tubule regeneration increased in the male and female 1,000 and 2,000 mg/L groups; increases in the 2,000 mg/L group significant
- Dose related increase in tungsten in blood and urine in all groups
- Urine xanthine/creatinine ratios significantly increased in all groups

Results: Subchronic Rat Studies Contd.

- Negative in micronucleus assay (male and female rats and mice) and bacterial mutagenicity assays (TA100, TA98, and *E. coli* WP2 *uvrA* pKM101, ± S9 mix)

- Increased DNA damage in comet assay observed in liver cells from male and female rats and in liver and ileum cells from male mice
  - Increases in DNA damage were not detected in peripheral blood leukocytes from male and female rats or mice, in ileum cells from female rats, in liver cells from female mice, or in kidney cells from male and female mice

Results: Subchronic Mice Studies

- No significant difference in body weight in any group at study termination

- Blood tungsten concentrations increased proportionally with exposure concentration; no observed sex difference.

- Kidney was the only target organ of toxicity
  - incidences of renal tubule regeneration higher in 1,000 and 2,000 mg/L males and females compared to respective vehicle controls; significant only in males

Dose – Selection Rationale for Chronic Studies

Rats: Significant decreases in body weight gain in rat dams during the lactation phase, and reductions in final mean body weight in weaned pups, informed the decision to lower the top exposure concentration in rats to 1,000 mg/L

Mice: No dose limiting toxicity in the subchronic studies informed decision to expose mice to up to 2,000 mg/L

Chronic Studies

Rats

Chronic Studies: Design

	Rats	Mice
Duration	GD6-PND21 + 2 years	2 years
Concentration (mg/L)	0, 250, 500, 1000	0, 500, 1000, 2000
Number of animals (per sex per concentration)	50 (core); 40 (special study)	50 (core); 40 (special study)
Interim Evaluations	3, 6, 12, 18 months (urine, blood, liver, kidney)	3, 6, 12, 18 months (urine blood, kidney, liver)
Endpoints	Survival Body Weights Water Consumption Clinical Observations Histopathology	Survival Body Weights Water Consumption Clinical Observations Histopathology

Results: Chronic Rat Studies- Perinatal Phase

- No significant effects on dam body weight or water consumption during gestation or lactation in any groups.

- No exposure-related effects noted on pregnancy status, maternal survival or number of dams that littered at any of the exposure concentrations tested

Rat Chronic Studies- Interim Evaluations

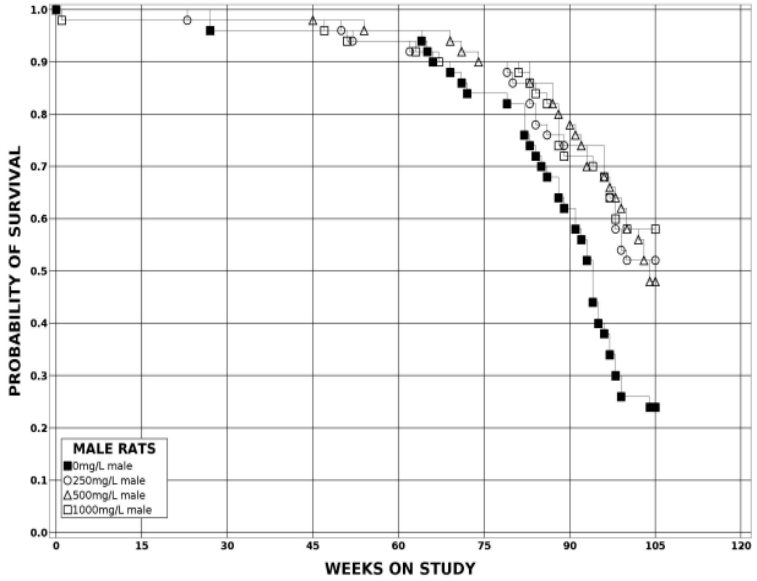
- Plasma and urine tungsten concentration increased with exposure concentration; no change with duration of exposure
- In kidney, tungsten concentration increased with exposure concentration and duration
    - Kidney/plasma ratio > 1 demonstrating retention of tungsten in kidney
- No observed sex differences in plasma, urine and kidney, tungsten concentrations

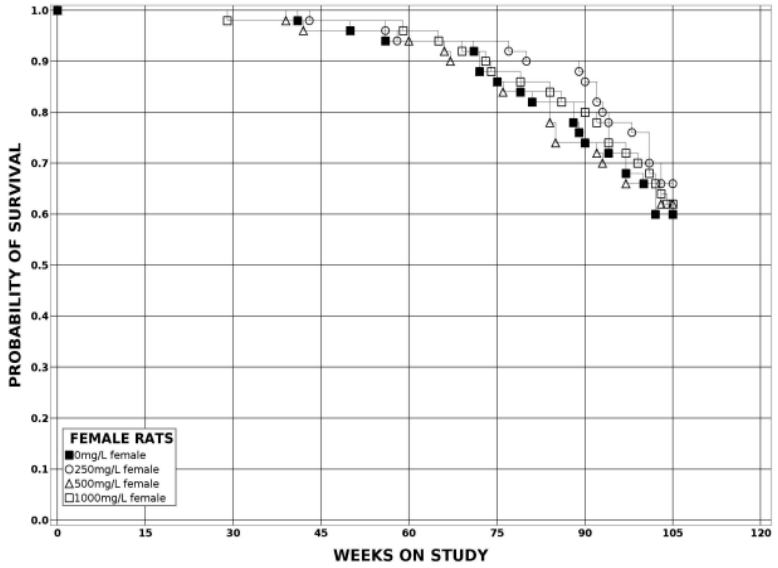
Public Health Context

- Plasma tungsten conc. in current studies following exposure to 250 mg/L tungstate in male rats and male mice are approximately **18,000** and **8,500** times higher, respectively, compared to humans (Bocca et al.., 2010)

- Urinary tungsten conc.in male rat exposed to 250 mg/L ST in these studies are **>**1,000,000 the urinary concentrations reported by a National Health and Nutrition Examination Survey program (2015–2016) (Lemus et al., 2015; CDC, 2013)

Chronic Rat Studies: Survival

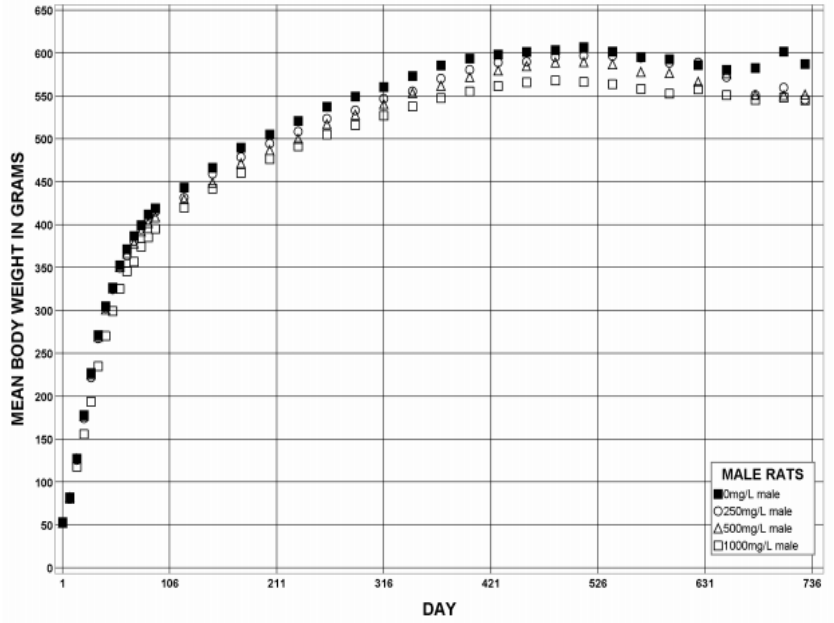


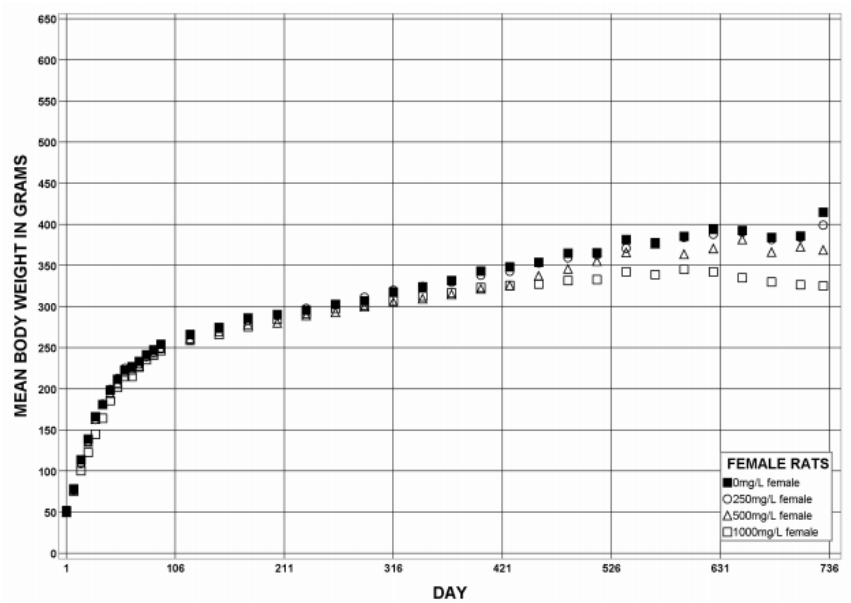


Survival significantly higher in exposed male rats compared to vehicle controls

No significant change in females

Chronic Rat Studies- Body Weight





No significant change in males

Females- mean body weights of the 500 mg/L and 1,000 mg/L groups - 88.9% and 78.5% of vehicle controls

Chronic Rat Studies: Thyroid Gland

Females	HC	0 mg/L	250 mg/L	500 mg/L	1000 mg/L
THYROID GLAND, C-CELL	HMB, I3C, PCTFT, RFR	50	50	49	50
Adenoma	10.78 ± 8.16% (4-22%)	5 (10%)	13 (26%)	13 (27%)*	8 (16%)
Carcinoma	0.5 ± 1% (0-2%)	2 (4%)	2 (4%)	2 (4%)	4 (8%)
Carcinoma or Adenoma	11.28 ± 8.16% (4-22%)	7 (14%)	15 (30%)	14 (29%)	11 (22%)
C-cell Hyperplasia		*14 [2.5]*	*13 [1.8]*	*9 [2.0]*	*12 [21.8]*

Chronic Rat Studies: Kidney

Males	0 mg/L	250 mg/L	500 mg/L	1000 mg/L
KIDNEY	50	50	50	50
Renal Tubule - Inflammation, Suppurative	*25** [1.2]*	*33 [1.3]*	*35 [1.3]*	*41** [1.6]*
Renal Tubule - Regeneration	*0*	*1 [2.0]*	*0*	*0*

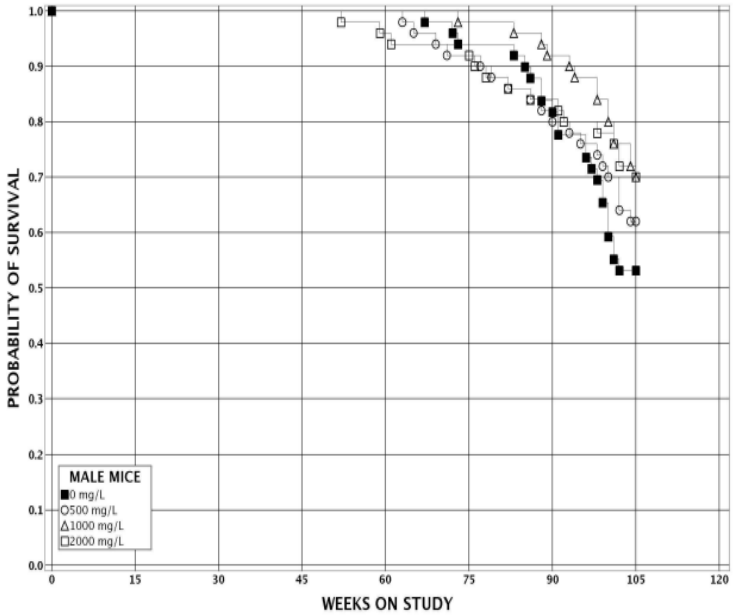
Females	0 mg/L	250 mg/L	500 mg/L	1000 mg/L
KIDNEY	50	50	50	50
Renal Tubule - Inflammation, Suppurative	*8** [1.0]*	*9 [1.0]*	*6 [1.0]*	*19* [1.1]*
Renal Tubule Regeneration	*0**	*0*	*0*	*18** [1.8]*

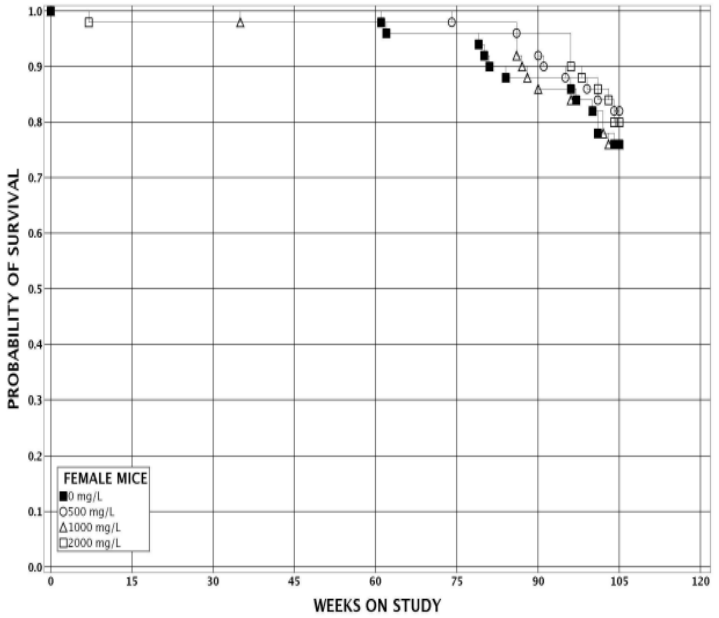
*P≤0.05; **P≤0.01

Chronic Studies

Mice

Chronic Mice Studies: Survival

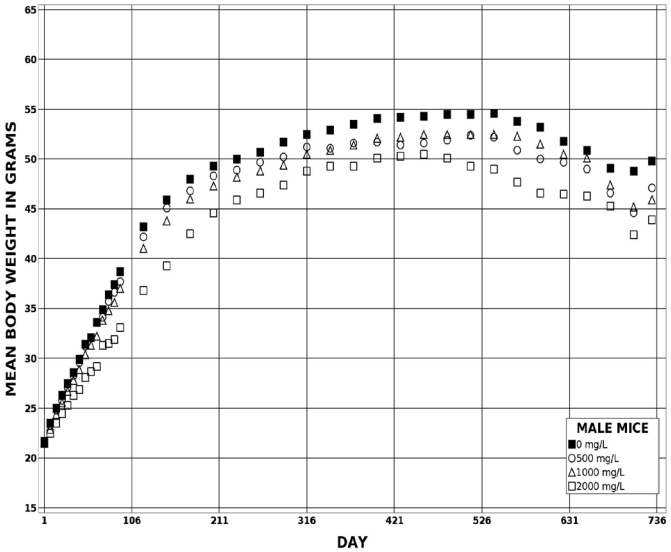


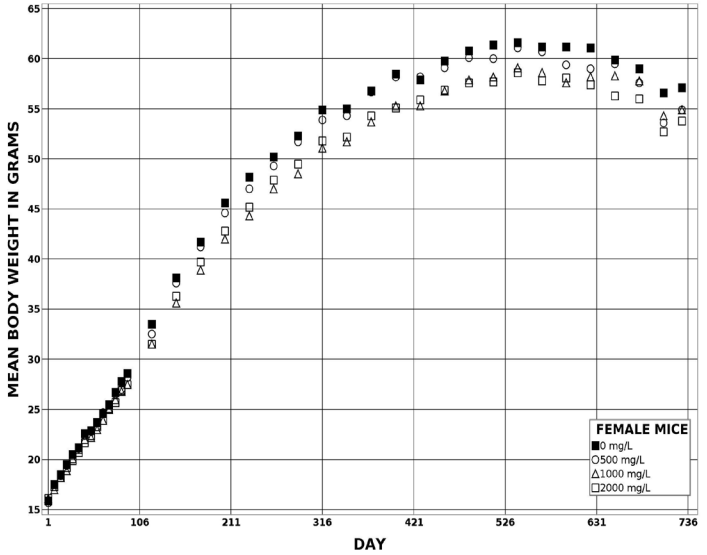


More male mice in the exposed groups survived to study termination compared to vehicle control; however, differences not statistically significant

No significant difference in females

Chronic Mice Studies: Body Weight





12% Decrease mean body weight gain in high dose males

No significant difference in females

Mice Chronic Studies- Interim Evaluations

- In mice, there was an increase in plasma, kidney, and urine tungsten concentrations with exposure concentration with a trend toward decreasing concentration with increasing exposure duration
  - Kidney/plasma ratios > 1 demonstrating retention of tungsten in kidney

- No observed sex difference in plasma, urine, or kidney tungsten concentration

Chronic Mouse Studies: Kidney

Males	Historical Controls	0 mg/L	500 mg/L	1000 mg/L	2000 mg/L
KIDNEY		50	50	50	50
Renal Tubule Adenoma	0.18 ± 0.6% (0-2%)	0	0	1 (2%)	0
Renal Tubule Carcinoma	0.36 ± 1.21% (0-4%)	0	0	0	2 (4%)
Renal Tubule Regeneration		*2** [1.0]*	*21** [1.4]*	*32** [1.4]*	*38** [1.6]*

Females	0 mg/L	500 mg/L	1000 mg/L	2000 mg/L
KIDNEY	50	50	50	50
Renal Tubule Regeneration	*0***	*1 [3.0]*	*7** [1.1]*	*7** [1.3]*

Conclusion: Rats

Male

- *No evidence of carcinogenic activity* at 250, 500, and 1,000 mg/L
- Exposure to sodium tungstate dihydrate in drinking water caused increased incidences of nonneoplastic lesions in the kidney of male rats

Female

- *Equivocal evidence of carcinogenic activity*
  - Increased incidences of C-cell adenoma or carcinoma (combined) of the thyroid gland
- Exposure to sodium tungstate dihydrate in drinking water caused increased incidences of nonneoplastic lesions in the kidney and uterus of female rats

Conclusion: Mice

Male

- *Equivocal evidence of carcinogenic activity*
  - Occurrences of renal tubule adenoma or carcinoma (combined) in exposed animals.
- Exposure to sodium tungstate dihydrate in drinking water caused increased incidences of nonneoplastic lesions in the kidney, testes, and bone marrow of male mice

Female

- *No evidence of carcinogenic activity* at 500, 1,000, and 2,000 mg/L
- Exposure to sodium tungstate dihydrate in drinking water caused increased incidences of nonneoplastic lesions in the kidney and spleen of female mice.

Questions?

Chronic Rat Studies: Uterus

Uterus

Females	HC	0 mg/L	250 mg/L	500 mg/L	1000 mg/L
UTERUS	HMB, I3C, PCTFT, RFR	50	50	50	50
Adenoma	0	0	0	0	1 (2%)
Adenocarcinoma	3.83 ± 4.33% (0-10%)	3 (6%)	0	2 (4%)	5 (10%)
Atypical Hyperplasia		*4 [2.3]*	*7 [1.4]*	*19** [1.7]*	*8 [2.3]*
Cyst		*0**	*0*	*0*	*3*

No significant differences in adenocarcinomas; incidences within historical control range

2-Year: Liver

*P≤0.05; **P≤0.0

Females	Historical Controls	0 mg/L	500 mg/L	1000 mg/L	2000 mg/L
LIVER	August 2017	50	50	50	50
Hepatocellular Adenomas (includes multiples)	18.67 ± 7.2% (6-28%)	11 (22%)	19 (38%)	11 (22%)	10 (20%)
Hepatocellular Adenoma, Multiple	-	5 (10%)	10 (20%)	7 (14%)	3 (6%)
Hepatocellular Carcinomas (includes multiples)	10.45 ± 4.93% (4-20%)	2 (4%)	8 (16%)	4 (8%)	3 (6%)
Hepatocellular Carcinoma, Multiple	-	0	0	1 (2%)	0
Hepatocellular Adenoma or Carcinoma	26.55 ± 8.77% (8-40%)	13 (26%)	24* (48%)	14 (28%)	13 (26%)
Eosinophilic Focus		*8*	*17**	*16*	*10*
Focal Inflammation		*14 [1.0]*	*24* [1.0]*	*21 [1.0]*	*23 [1.0]*

No evidence" based on common background tumor with no dose response, significance only at low dose.