The potential reproductive, neurobehavioral and systemic effects of soluble sodium tungstate exposure in Sprague–Dawley rats

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A B S T R A C T

The debate on tungsten (W) is fostered by its continuous usage in military munitions. Reports demonstrate W solubilizes in soil and can migrate into drinking water supplies and, therefore, is a potential health risk to humans. This study evaluated the reproductive, systemic and neurobehavioral effects of sodium tungstate (NaW) in rats following 70 days of daily pre-and postnatal exposure via oral gavage to 5, 62.5 and 125 mg/kg/day of NaW through mating, gestation and weaning (PND 0–20). Daily administration of NaW produced no overt evidence of toxicity and had no apparent effect on mating success or offspring physical development. Distress vocalizations were elevated in F1 offspring from the high dose group, whereas righting reflexes showed unexpected sex differences where males demonstrated faster righting than females; however, the effects were not dose-dependent. Locomotor activity was affected in both low and high-dose groups of F1 females. Low-dose group showed increased distance traveled, more time in ambulatory movements and less time in stereotypic behavior than controls or high dose animals. The high-dose group had more time in stereotypical movements than controls, and less time resting than controls and the lowest exposure group. Maternal retrieval was not affected by NaW exposure. Tungsten analysis showed a systemic distribution of NaW in both parents and offspring, with preferential uptake within the immune organs, including the femur, spleen and thymus. Histopathological evidence suggested no severe chronic injury or loss of function in these organs. However, the heart showed histological lesions, histiocytic inflammation from minimal to mild with cardiomyocyte degeneration and necrosis in several P0 animals of 125 mg NaW dose group. The result of this study suggests that pre and postnatal exposure to NaW may produce subtle neurobehavioral effects in offspring related to motor activity and emotionality.

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Introduction

Military use of tungsten (W) as a replacement for lead and depleted uranium in bullets and munitions has been significantly increased (Kerley, et al., 1996). However, reports demonstrate that tungsten solubilizes in soil and can migrate into ground water (Dermatas et al., 2005). Therefore W is a potential health risk to humans from contact with contaminated soil or water or bioaccumulation of tungsten via food sources. Literature indicates that soluble tungsten compounds are readily absorbed following oral exposure in both humans and laboratory rats (ATSDR, 2005; Ballou, 2002; LeLamer et al., 2000). In rat, it has been shown that the embedded tungsten alloy (W/Ni/Co) pellets caused aggressive metastatic tumors in 16–20 weeks post implantation study (Kalinch, et al., 2005). This metastatic tumor was confirmed in 6 month WA (W/Ni/Co) implanted rats by another group from US Army Center for Health Health and Promotion (USACHPPM) (Roszell et al., 2008). In addition, USACHPPM (McCain et al., 2008) demonstrated that 90 day NaW oral dose exposure produced toxic sign in the kidney tubules with cellular debris with or without hypoxemia in the epididymis specifically at higher doses (125 and 250 mg). Furthermore, the developing brain may also be at risk as it has been demonstrated that systemic tungsten readily crosses the placenta into the fetuses of pregnant rats (Nadeenko et al., 1978; Wide et al., 1986; ATSDR, 2005) and is present in the milk of exposed dams. Additionally, unspecified morphological changes were detected in the cerebral cortices of rats exposed orally to sodium tungstate for 8 months and appeared to correlate with neurobehavioral perturbations (Nadeenko, 1966; Nadeenko et al., 1978). However, there was no sufficient information to fully typify the reproductive and behavioral alterations due to the impact of tungsten exposure. The present study was conducted to screen neurobehavioral effects resulting from chronic oral sodium tungstate exposure in adult male and females and their offspring.

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Methods

A small battery of neurobehavioral tests were selected specifically to assess neurological capacities in both the exposed dams and their offspring. The methodologies used in this study were based on USEPA Guideline OPPTS 870.3650. Each male and female Sprague–Dawley rat (8 weeks old) at the end of the two-week acclimation period was randomly assigned to one of the four groups (vehicle control, 5, 62.5 and 125 mg/kg/day) (Table 1). Dosing continued for 70 days and encompassed mating following 14 days of treatment, that continued through the 14 day mating period, the 22 day gestational period, and through to postnatal day (PND)20. In order to ensure a total of 70 day exposure, adults were dosed for any additional days necessary following weaning. Prenatal pup exposure was from sodium tungstate crossing through the placenta and postnatal exposure was indirect via dams’ milk. Offspring (F1 generation) were monitored until PND70. On PND1 (day of parturition = PND 0), the number of pups in each litter was recorded. Litters were culled on PND4 to eight pups, and litters were weighed. Selection of pups was pseudo-random to maintain a 4 male/4 female ratio whenever possible, and all pups remained with their biological mothers. Inductively coupled plasma mass spectrometry (ICP-MS) was used to measure tungsten concentrations in brain tissue of adults and pups from the control and highest dose groups only and in dam mammary secretion in all control, low and high dose P1 groups. Adults (10 of each sex) from each dose group were necropsied on day 70, and pups (one male/one female) from each litter were necropsied at PND20 and PND70. Weight of pregnant dams and gestation length were recorded as was the litter size. Litters were culled on PND4 to maintain a 4 male/4 female ratio whenever possible, and all pups remained with their biological mothers. Animals were observed for clinical signs of toxicity throughout the exposure period, although none were noted. Additionally, no deaths were recorded for the adult females or males at the doses tested.

Neurobehavioral tests. The following neurobehavioral test batteries were performed on pups and adult females after exposure to NaW. The righting reflex and separation distress were done on PD4 and PND7, respectively (Pellis et al., 1991; Bekkedal et al., 1999; Hahn and Lavooy, 2005). The adult females were tested for maternal retrieval latency when pups were age PND2 (Hahn and Lavooy, 2005), and spontaneous locomotor activity (SLA) on post-dosing day 7. All statistical testing was performed using ANOVA with repeated measures factors. Tukey’s HSD analysis was used to evaluate pair-wise comparisons.

Table 1

| Dosage Paradigm of Sodium Tungstate in P0 Generation Rats and F1 Generation. |
|---|---|---|---|
| Control (water) | Low Dose | Medium Dose | High Dose |
| 5 mg/kg/day | 5 mg/kg/day | 125 mg/kg/day | 125 mg/kg/day |
| n=40 males | n=40 males | n=40 males | n=40 males |
| n=40 females | n=40 females | n=40 females | n=40 females |

Fig. 1. Weight gain of the F1 females and males after 70 consecutive days of oral dosing with NaW (5, 62.5 or 125 mg/kg/day) or water. (Bottom Left) and (Bottom Right). Gestational length (days) (Bottom Left) and weight gain (grams) (Bottom Right) of the pregnant P0 females after 70 consecutive days or oral dosing with NaW (5, 62.5, 125 mg/kg/day) or water.
Immunohistochemistry. At the end of experimentation, rats were perfusion-fixed with 4% paraformaldehyde. Various organ tissues (heart, spleen, kidney, liver, lungs, brain, testes, ovaries, thymus, bone, gastrointestinal tract, etc.) were carefully removed and postfixed in 4% paraformaldehyde no longer than 24 h. After dehydration, tissues embedded in paraffin and 40 μm coronal sections were made on glass slides for histological evaluation using hematoxylin and eosin (H&E) stain.

Results

The study results indicate that a significant difference was found for one of the treatment groups on measures of reproductive toxicity. In the dams, particularly 125 mg dose group animals showed longer gestational period when compared to control, 5 and 62.5 mg groups. Sodium tungstate treatments did not have an effect on average gestational weight gain in adults and offspring (Fig. 1).

Tungstate ion concentrations in the male and female adult and pup brains after sodium tungstate exposure were significantly greater in the high dose (125 mg) treated rats than control (data not shown). Similarly, in dam milk secretions tungstate ion concentrations was significantly greater in the 125 mg treatment group than in the low dose group or controls (data not shown). Further, we also observed increased concentration of tungstate ion distribution in other major organs like heart, spleen, kidney, thymus, testes, lungs, liver, femur bone and gastrointestinal regions in both male and female treated adult and pups (Fig. 2).

Fig. 2. (Upper Left) and (Upper Right). Concentration of W in the major organs of F1 males (left) and females (right) after 20 consecutive days of oral dosing with NaW (62.5, 125 mg/kg/day) or water. Asterisk denotes significant NaW compared to control (p < 0.05). 5 mg/kg not shown due to insufficient data. By PND70, W levels were below detectable limits. Data not shown. (Bottom Left) and (Bottom Right). Concentration of W in the major organs of P0 males (Left) and females (Right) after 70 consecutive days of oral dosing with NaW (62.5 or 125 mg/kg/day) or water. Asterisk denotes significant NaW compared to control (p < 0.05). 5 mg/kg not shown due to insufficient data.

Fig. 3. Neurobehavioral testing in pups and dams following sodium tungstate exposure. Emotionality of the pups was affected only at the high dose. In the dams, opposing exposure effects were observed in the low and high dose groups related to locomotor activity and exploration.

Neurobehavioral test

A significant effect of tungstate exposure on spontaneous locomotor activity was detected especially with low dose treated dams. Compared to control and the high dose treated animals, the low dose treated dams spent more time on ambulatory time and distance traveled and less time in stereotypies. On the contrary, in high dose treated dams less time resting and more time in stereotypic movements than the controls or low dose group were markedly observed (Fig. 3). For righting reflex, a significant sex effect was found on male where males were faster than females. However, there was no dose related effect on this activity (Fig. 3). Further, on ultrasonic distress vocalization test, pups showed dose-related effects during 60 s time period. The high dose treated pups exhibited more vocalization than control and 5 mg groups (Fig. 3). Maternal retrieval test revealed that sodium tungstate exposure to dams had no effect on latency in both treatment groups (Fig. 3). This result indicates that both 5 and 125 mg exposed male (not female) pups motor reflex were affected due to sodium tungstate. However, only high dose exposed males were affected by emotionality. In the dams, exposing exposure effects were observed in the low and high dose groups related to locomotor activity and exploration (data not shown).

Histopathology

As summarized in the Table 2, histopathological examination showed no severe chronic injury in the organs tested. However, the heart showed histological lesions, histiocytic inflammation from minimal to mild with cardiomyocyte degeneration and necrosis in several P0 animals of 125 mg NaW dose group (Fig. 4).

Discussion

Overall, the results suggest that sodium tungstate exposure to pre- and postnatal rats may produce slight neurobehavioral effects related to motor activity and emotionality in offspring. Furthermore, this study results are consistent with previous reports that prenatal and early postnatal exposures to tungsten affect neurobehavioral development (Nadeenko and Lenchenko, 1977; Nadeenko et al., 1978). In addition, we showed hereby that while sodium tungstate did not cause significant change in gestation length and weight gain in adult females, we observed significant alteration in maternal behavior. The ultrasonic distress test revealed that the high dose treated animal demonstrated greater number of vocalization when dams were separated from littermates. The average righting reflex latency test on pups showed difference in their activity among sex in which males demonstrated faster righting than females, but the effects were not dose-dependent. The neurobehavioral effects observed in pups may be secondary effects of sodium tungstate exposures on CNS function by involving possible cleavage products of this metal that can be transported into the brain like other metals such as lead (Qian et al., 2005) and produce cellular toxicity. Previous reports documented that the brain is one of the vulnerable target organs due to exposure to tungstate and its effects are reflected through behavioral response (Nadeenko, 1966; Wase, 1956; Nadeenko and Lenchenko, 1977). Though our results provide evidence for neurological effects in F1 offspring, neurological lesions could not be predicted based on the toxic effects of sodium tungstate. In the present study rats exposed to different dose of NaW did not show dose response in pups or dams and provided no indication of brain susceptibility. However, these results are consistent with the previous report that indicates sodium tungstate exposure caused reflexive deficit results in neurobehavioral perturbations (Nadeenko, 1966).

From this study we also found significant difference in the locomotor activity with dose following challenge with NaW in P1 dams. Low dose treated dam explored increased activity in distance traveled while high dose groups exhibited decreased stereotypies. The increased exploration is evident from increased ambulatory time and distance traveled. This change from baseline may represent hyperactivity or some other diminution of affective inhibition. Elevated gross motor activity in the low dose group is in opposition to the increased time spent in stereotypic movements in the high dose group. This increased time in

![Normal Heart](A)

![Minimal Histocytic Myocarditis](B)

![Mild Histocytic Myocarditis](C)
stereotypies for the high dose exposure corresponds to a reduction in time spent resting in the high dose group. The altered stereotypical behavior suggests a subtle effect in the motor system that is not apparent in the measures of gross motor movements in the open field or in the reflexive acoustic startle or prepulse inhibition responses. Though this effect appears to be inconsistent, hyperactivity noted at low dose seem to be linked to the progression of the stereotypies at the higher dose. This suggests that further investigation on locomotor behavior is warranted based on lack of dose–response relationship.

Pathological examination revealed that no treatment related deaths or histopathological changes in any organs except heart were observed in rats following sodium tungstate administration up to 125 mg NaW by oral gavage for 70 days, perhaps due to low intestinal absorption of NaW. Histopathological evaluation revealed minimal to mild lesions, histiocytic inflammation with cardiomyocyte degeneration and necrosis within the heart in several P0 animals of higher dose group. Hence, an additional research is required in F2 generation individuals to characterize the neurotoxicity and cardiac pathology of sodium tungstate.

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