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90-Day subchronic toxicity study of sodium molybdate dihydrate in rats

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ABSTRACT

This study investigated the subchronic toxicity of molybdenum (Mo) in Sprague–Dawley rats given sodium molybdate dihydrate in the diet for 90 days at dose levels of 0, 5, 17 or 60 mg Mo/kg$_{bw}$/day. The study complied with OECD Test Guideline (TG) 408, with additional examination of estrus cycles and sperm count, motility, and morphology from OECD TG 416. The overall no-observed-adverse-effect level was 17 mg Mo/kg$_{bw}$/day, based on effects on body weight, body weight gain, food conversion efficiency and renal histopathology (females only) at 60 mg Mo/kg$_{bw}$/day. No treatment-related adverse effects on reproductive organ weights or histopathology, estrus cycles or sperm parameters were observed at any dose level. No adverse effects were observed in the high dose animals after the 60-day recovery period, with the exception that male rats did not fully recover from reduced body weight. Serum blood, liver and kidney samples were analyzed for molybdenum, copper, zinc, manganese, iron, cobalt and selenium; high levels of molybdenum and copper were found in the serum, blood, liver and kidneys of rats treated with 60 mg Mo/kg$_{bw}$/day. In conclusion, the LOAEL and NOAEL for molybdenum were determined to be 60 and 17 mg Mo/kg$_{bw}$/day, respectively.

1. Introduction

Molybdenum is an essential element for humans, as well as for animals and plants (Institute of Medicine, 2001). In humans and other mammals, molybdenum is a key constituent of at least three important enzymes: sulfite oxidase, xanthine oxidase and aldehyde oxidase (Kisker et al., 1997). Trace levels of molybdenum are found in a wide variety of foods, and human exposure to molybdenum may occur via the diet, drinking water and occupational exposure from mining operations and industrial uses.

Various countries have issued recommendations on adequate intake levels and minimum dietary requirements of molybdenum (Turnlund and Friberg, 2007). For example, the USA National Academy of Sciences Food and Nutrition Board (Institute of Medicine, 2001) has estimated the average requirement of molybdenum for adults is 34 μg/day, corresponding to about 0.5 μg/kg$_{bw}$/day for a 70-kg adult, whereas the British Expert Committee on Vitamins and Minerals has estimated molybdenum requirement in the range 100–300 μg/day (1.4–4.3 μg/kg$_{bw}$/day) (Turnlund and Friberg, 2007).

Human deficiencies of molybdenum have not been linked to inadequate dietary intake (Nielson, 1999; Turnlund and Friberg, 2007; European Food Safety Authority (EFSA), 2010). Reports of molybdenum deficiency in humans have been limited to genetic defects that interfere with the molybdenum cofactor's ability to activate molybdoenzymes and to one case of feeding molybdenum-free total parenteral nutrition (EFSA, 2010).

The toxicity of molybdenum compounds in humans has been observed to be low, and in general, soluble molybdenum compounds (e.g., sodium molybdate dihydrate) are more toxic than insoluble compounds (Vyskocil and Viau, 1999). The symptoms of molybdenum toxicity in humans are reported to resemble those of copper deficiency, and supplemental copper usually reverses them. High dietary levels of molybdenum have been shown to affect copper metabolism in humans, resulting in an increase in copper excretion and elevated levels of plasma copper (Deosthale and Gopalan, 1974). Thiomolybdate is a potent copper chelating agent and has been used in humans for the treatment of Wilson's disease,

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a condition related to high body copper levels (Suzuki and Ogura, 2000; Quagraine and Reid, 2001).

The toxicity of high dietary levels of molybdenum to cattle and sheep has been known for many years (Ward, 1994). Excessive molybdenum causes a condition known as "teart", or molybdenosis, which is characterised by scouring (diarrhea), rapid loss of weight and condition, the development of harsh, discolored coats, and ultimately death if not treated. Toxicity occurs in cattle on pastures with relatively high molybdenum contents of 20–100 ppm compared with 3–5 ppm on "normal" pastures (Turnlund and Friberg, 2007), and the toxicity has been shown to be associated with reduced bioavailability of copper due to the high levels of sulfur in the rumen which interacts with molybdenum to form thiomolybdates (Ward, 1994).

Toxicity studies in rats have suggested that molybdenum can induce testicular damage (Pandey and Singh, 2002), as well as interfere with estrus cycles and cause developmental toxicity (Fungwe et al., 1990). However, these studies have significant limitations, and these findings have not been confirmed in other studies.

The purpose of the present study was to assess the subchronic toxicity of sodium molybdate dihydrate when administered orally (adjusted dietary administration) to male and female rats for 90 days, followed by a recovery period of up to 60 days for the control and high dose groups. The study was performed in accordance with OECD Test Guideline 408 (OECD, 2011). In addition, in view of the observations on testes and estrus cycles in the published literature, it was decided to include examination of estrus cycles, analysis of various sperm parameters and detailed histopathology of reproductive organs. Serum, whole blood and tissue levels of molybdenum, copper and other trace elements were also measured.

2. Materials and methods

2.1. Test substance

Sodium molybdate dihydrate ($Na_2MoO_4{\cdot}2H_2O$; Lot No. 43006L; purity >99%) was supplied by Climax Molybdenum Marketing Corporation (Phoenix, AZ). The test substance was selected as a source of molybdate ion $[MoO_4]^{2-}$ that would be representative of soluble molybdenum(VI) compounds that give rise to molybdate ions under physiological conditions. Sodium molybdate dihydrate may be expressed in terms of molybdenum equivalents by dividing by 2.5 (the ratio of the molecular weights of sodium molybdate dihydrate and molybdenum is 242/95). For example, 50 mg of sodium molybdate dihydrate is equivalent to 20 mg of molybdenum. The doses used for this study are expressed herein as mg Mo/kg_{bw}/day.

For dietary administration, the test substance was admixed into Certified Rodent Diet (No. 2016C; Harlan Teklad, Madison, WI). Analysis of the diet did not reveal any contaminants which would be expected to interfere with the studies. For the 90-day study, dietary dose formulations were prepared once weekly for the first 4 weeks of the study, then every other week for the rest of the treatment period to achieve the target dose levels in mg Mo/kg_{bw}/day. Dietary dose formulations were stored at room temperature under low humidity conditions when not in use. Homogeneity of the dietary dose formulations was confirmed, and stability was confirmed to be at least 5 weeks. Analysis of this basal control diet showed that the mean content of molybdenum and copper was 906.5 μg/kg and 14.23 mg/kg dry weight, respectively. Based on this concentration of molybdenum in the control diet and the measured rate of food consumption, the control group animals received a mean exposure to molybdenum of approximately 0.08 mg Mo/kg_{bw}/day.

2.2. Animals and husbandry

Male and female outbred albino rats (Crl:CD®(SD)IGS BR, VAF/Plus® Sprague–Dawley derived) were used. The animals used for the 90-day study were supplied by Charles River Laboratories, Kingston, NY, USA. They were received at the test facility at approximately 7 weeks of age and stabilized for 2–2.5 weeks, during which they were examined for suitability for the study, before commencing treatment. At the start of dosing, at approximately 9 weeks of age, average body weight of the males was 338.4 g (range 309.8–377.6 g) and of the females was 229.6 g (range 187.9–263.5 g).

Animals were individually housed in elevated, stainless steel, wire mesh cages, maintained in rooms under a 12 h light/dark cycle, with a daily average temperature range of 19–23 °C and a daily average relative humidity range of 34–49%. Feed was available without restriction. Tap water (New Jersey-American Water Company, Cherry Hill, NJ) was available without restriction via an automated watering system. Water analysis did not reveal any contaminants which would be expected to interfere with the study.

2.3. Study design

The 90 day toxicity study was performed at Huntingdon Life Sciences (HLS; East Millstone, NJ) in compliance with Good Laboratory Practice (GLP) and in accordance with OECD Guideline 408 (OECD, 2011). In addition, in view of the observations on testes and estrus cycles in the published literature, it was decided to include examination of estrus cycles, analysis of various sperm parameters and detailed histopathology of reproductive organs as described in the OECD Guideline 416 for a two-generation reproduction toxicity study.

Sodium molybdate dihydrate was given by dietary administration to male and female rats for 91 and 92 consecutive days, respectively. Rats were assigned to groups using a computerized random sort program so that body weight means for each group were comparable as follows: Groups 1 (control) and Group 4 (high dose) contained 20 animals/sex/group, and Groups 2 (low dose) and 3 (middle dose) contained 10 animals/sex/group. At the end of the 90-day treatment period, 10 animals/sex/group were necropsied. The remaining 10 animals/sex/group in Groups 1 and 4 were continued on untreated diet for up to 60 days. At the end of the recovery period, they were necropsied.

In the 90-day study, the target doses for Groups 1, 2, 3 and 4 were 0, 5, 17 and 60 mg Mo/kg_{bw}/day, respectively. The dose levels were selected based on the results of a 28-day range finding study, conducted at the same laboratory, in which sodium molybdate dihydrate was given to groups of 5 rats/sex/group in the diet or by gavage (once or twice daily) for 28 consecutive days at dose levels of 0, 4, or 20 mg Mo/kg_{bw}/day. Also, in the range-finder study, blood was taken from the jugular vein from 5 rats/sex/group/time point on day 27 (dietary administration groups) or day 28 (gavage administration groups) at five time intervals in order to study blood levels of molybdenum.

There was no mortality or treatment-related clinical signs in any of the groups of rats in the range-finder study. Decreases in body weight (8% decrease on day 28; not statistically significant; $n = 5$) and body weight gain (19% decrease on day 28; $p < 0.05$ at most time intervals; $n = 5$) were observed among males given the high dose (20 mg Mo/kg_{bw}/day) in the diet. In females, no effect on body weight or body weight gain was observed at any dose level. There were no convincing treatment-related effects on organ weights or macroscopic or microscopic findings.

Since little evidence of toxicity was found in the range finder study, it was decided to use a higher dose range in the 90-day

study of 0, 5, 17 and 60 mg Mo/kg_{bw}/day. The top dose level of 60 mg Mo/kg_{bw}/day is at least 20,000 times the normal human dietary exposure of 1–2 μg Mo/kg_{bw}/day (Turnlund and Friberg, 2007). The dietary route of exposure was chosen because this is the normal route of human exposure and the diets were shown to be palatable in the 28-day study, producing substantial dose-related increases in serum molybdenum levels.

2.4. Clinical observations

All animals were observed in their cages twice daily for mortality and general condition. During the treatment period, all animals were observed for signs of toxic and pharmacological effects at least twice daily. Animals were removed from their cages and examined once pretest and weekly during the study period (except examinations were performed twice during the first week of the recovery period). Examinations included observations of skin and fur, eyes and mucous membranes, respiratory and circulatory effects, autonomic effects such as salivation, central nervous system effects, including tremors and convulsions, changes in the level of motor activity, gait and posture, reactivity to handling or sensory stimuli, grip strength, and stereotypies or bizarre behavior.

2.5. Ophthalmic examination

An ophthalmic examination was performed on all animals at pretest and at the termination of the treatment period. The cornea, anterior chamber, lens, iris, vitreous humor, retina, and optic disc were examined by indirect ophthalmoscopy.

2.6. Body weight and food consumption

Animals were removed from their cages and weighed twice pretest, weekly during the study and terminally (after fasting) just prior to necropsy. Food consumption was measured pretest, at Days 2, 4, and 7 during the first week of treatment, and weekly (at 6 day intervals) thereafter. The amount of food consumed over a 6-day period during the week was used to determine feed concentration calculations for the following week for the first 4 weeks and every other week during the rest of the study.

2.7. Hematology and blood chemistry

Blood samples were collected via the jugular vein (unanesthetized or lightly anesthetized with isofuorane) or orbital sinus (lightly anesthetized with isofluorane) using dipotassium EDTA (for hematological evaluation) or sodium citrate (for coagulation studies) as an anticoagulant or no anticoagulant (for clinical chemistry). Animals were fasted overnight prior to blood collection. The following hematological parameters were evaluated on a Bayer Advia 120 hematology analyzer: hemoglobin concentration, hematocrit, erythrocyte count, platelet count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, total leukocyte count, reticulocyte count, and differential leukocyte count. Coagulation parameters (prothrombin time and activated partial thromboplastin time) were determined on a mechanical clot detection system (STA Compact, Diagnostica Stago Products).

The following clinical chemistry parameters were determined on a Hitachi 917 chemistry analyzer: aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, blood urea nitrogen, creatinine, glucose, cholesterol, triglycerides, total protein, albumin, uric acid, total bilirubin, sodium, potassium, chloride, calcium, and inorganic phosphorus.

2.8. Vaginal cytology/estrus cycles

Daily vaginal smears were taken from each female at approximately the same time each day and stage of estrus determined for 3 weeks starting after completing 6 weeks of treatment. The overall pattern of each female was characterized as regularly cycling (having recurring 4–5 day cycles), irregularly cycling (having cycles with a period of diestrus longer than 3 days or a period of cornification longer than 2 days), or not cycling (having prolonged periods of either vaginal cornification or leukocytic smears). Cycle length was defined as the number of days from one estrus to the next. Incomplete cycles were not included in calculating the mean cycle length.

2.9. Sperm evaluation

Sperm evaluations were performed as outlined in OECD TG 416. All surviving male animals were processed for sperm counts and sperm motility at the terminal sacrifice and the recovery sacrifice using a Hamilton Thorne IVOS sperm analyzer. For sperm and spermatid counts, homogenized samples of the right caudal epididymis and the right testis, respectively, were stained and examined; for each stained preparation, 10 fields were counted. For sperm motility, the right vas deferens of all surviving males was excised and placed in a pre-warmed solution of phosphate buffered saline and 7.5% bovine serum albumin fraction V in Medium 199. After a swim-out period, motility was evaluated using at least 200 sperm and or five microscope field images. Two sperm morphology slides were prepared for all surviving males at terminal sacrifice and recovery sacrifice; approximately 200 sperm per animal within the 2 slides were evaluated.

2.10. Necropsy

All animals were fasted overnight prior to necropsy and euthanized by carbon dioxide inhalation followed by exsanguination. All animals were subject to macroscopic examination, which included external surface and all orifices, the external surfaces of the brain and spinal cord, the organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and neck, and the remainder of the carcass.

2.11. Organ weights

Absolute and relative organ weights (based on terminal body weights) were determined for the adrenal glands, brain, epididymides, heart, kidneys, liver, ovaries, pituitary gland, prostate gland and seminal vesicles, spleen, testes, thymus, thyroid/parathyroid glands, and uterus with cervix.

2.12. Histopathology

All organs and tissues obtained during necropsy were preserved. Slides of these organs and tissues were prepared and examined microscopically for all Group 1 (control) and Group 4 (high-dose) animals sacrificed at termination of the treatment period. In addition, the adrenal glands from males and the kidneys from females in Groups 2 and 3, sacrificed at termination of the treatment period, and from animals in Groups 1 and 4 sacrificed at the end of the recovery period were examined microscopically.

2.13. Elemental analysis of serum, whole blood, liver and kidney

Blood and serum samples were taken from 10 rats/sex/group and analyzed for molybdenum, copper, zinc, manganese, iron, cobalt and selenium at weeks 4 (serum only) and 12 (serum and

blood), as well as at 2 and 7 days after cessation of treatment for the recovery groups (serum only). Liver and kidney samples from 10 rats/sex/group were collected at the end of the treatment period and the recovery period, and they were flash frozen in liquid nitrogen and shipped on dry ice to the analytical laboratory for analysis of the same elements as the serum samples. Serum, whole blood, and tissues were analyzed for molybdenum and the other elements at Michigan State University (East Lansing, MI) using a validated inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7500ce) method. The same laboratory and instrument was also used to analyze the feed samples.

2.14. Statistical analysis

Continuous data, including body weights, food consumption, hematology and clinical chemistry parameters, organ weights and sperm analysis, were subjected to a Bartlett's test for variance homogeneity (Bartlett, 1937). If this was not significant at the 1% level, then parametric methods were applied using either Williams' test (Williams, 1971, 1972) or Dunnett's test (Dunnett, 1955, 1964). For non-continuous data, statistical tests were ranked and examined by the Kruskal–Wallis test (Kruskal and Wallis, 1952, 1953). If significant, the Wilcoxon rank sum test (Wilcoxon, 1945) was applied for comparison with the vehicle control group. For analyses of serum, whole blood and tissue levels of metals and other elements in the 90-day study, one-sided pairwise comparisons of each treatment group with their respective control were conducted using the R software package version 2.14.1 (R Development Core Team, 2011).

3. Results

3.1. Analysis of diets

Chemical analysis confirmed that the diets were of appropriate concentration of molybdenum and that homogeneous dietary mixtures were administered. Mean analytical concentrations of molybdenum in the diets were 91–102% of the nominal concentrations. Exposure to the test substance was generally comparable to the targeted dose levels, but with the males it was consistently less than intended and with the females consistently more than intended. Specifically, the actual mean intake of molybdenum at the nominal dose levels of 5, 17, and 60 mg Mo/kg_{bw}/day was 4.5, 15.1, and 54.8 mg Mo/kg_{bw}/day, respectively, among males and 5.4, 19.0, and 65.2 mg Mo/kg_{bw}/day, respectively, among females, giving overall mean intakes of 5.0, 17.1 and 60.0 mg Mo/kg_{bw}/day.

3.2. Mortality and clinical observations

One male in the group given 60 mg Mo/kg_{bw}/day was found dead on Day 47. There were no clinical signs prior to death and no macroscopic or microscopic findings to explain the cause of death. This single death was considered incidental and unrelated to the administration of the test substance. There was no mortality in any other groups. There were no treatment-related clinical signs or ocular abnormalities in any of the animals.

3.3. Body weight and food consumption

During the 90-day treatment phase, statistically significant decreases in body weight gain compared to controls were observed in males and females exposed to 60 mg Mo/kg_{bw}/day beginning as early as weeks 1 and 6, respectively (Figs. 1 and 2). At the end of the 90-day treatment period, the final body weights of males and females given 60 mg Mo/kg_{bw}/day were 15.1% ($p < 0.001$) and 5.6% (not statistically significant) less, respectively, than their respective control values. During the 60-day recovery phase, the males in the group given 60 mg Mo/kg_{bw}/day showed statistically significant increases in body weight gains at each weekly interval, but the absolute body weight was still significantly less than controls (by 9.5%; $p < 0.05$) at the end of the recovery phase. There were no treatment-related effects on body weight or body weight gain among the males or females given 5 or 17 mg Mo/kg_{bw}/day.

In males given 60 mg Mo/kg_{bw}/day, food consumption was statistically significantly decreased on numerous occasions throughout the treatment period. Otherwise, compared to controls, no consistent difference in food consumption was observed in any of the other groups of males or females receiving the test material. At 60 mg Mo/kg_{bw}/day, food conversion efficiency was significantly reduced during the treatment period, particularly among the males, suggesting that some interference with nutrition, as well as reduced food consumption, may have played a role in reduced body weight gain.

3.4. Hematology and clinical chemistry

There were no effects on hematological parameters, apart from a marginal ($\leqslant$1 s), statistically significantly shorter prothrombin times in males receiving 5, 17 or 60 mg Mo/kg_{bw}/day. These changes were not considered to be related to the administration of the test substance because they were slight, not dose-related, and not observed in females. No significant difference from controls was seen for activated partial thromboplastin time in any group of males or females.

The results of clinical biochemistry evaluation revealed statistically significant decreases in total protein and calcium at 60 mg Mo/kg_{bw}/day in males, but not in females. Creatinine and uric acid levels were statistically significantly reduced compared to controls among females at all dose levels, but a similar effect was not observed in males. The changes in clinical chemistry parameters were not considered to be treatment-related because they were small in magnitude, not dose-related, due to outliers in control animals, and/or were consistent with normal biological variability.

3.5. Estrus cycles and sperm evaluation

There were no test substance-related effects on vaginal cytology or estrus cycles when observed during weeks 7–9 of the treatment phase (Table 1). No significant differences were observed in the mean length of the cycle or the mean number of cycles among groups of females exposed to molybdenum compared to controls. One female with an irregular cycle was observed in the control group and in each treated group.

In males, no effect of treatment was observed on spermatid or sperm counts (Table 2), or on sperm morphology. No significant effect on overall sperm motility was observed at any dose level at the terminal sacrifice, although the 60 mg Mo/kg_{bw}/day males had a slight but statistically significant decrease in progressively motile sperm at the terminal sacrifice (59.0% vs. 69.4% in controls). This slight decrease in progressively motile sperm at the high dose was likely attributable to the control group having a value that approached the upper limit for this parameter among historical control groups (mean of 59.8 ± 16.2%) and was therefore not considered a test substance-related finding. The sperm morphological evaluation found no significant effect in any group on the percent normal sperm or the percent of individual abnormalities (i.e., decapitated, abnormal head, abnormal neck, abnormal tail, midtail blob). At the end of the recovery period, there was no significant effect on any sperm parameter.

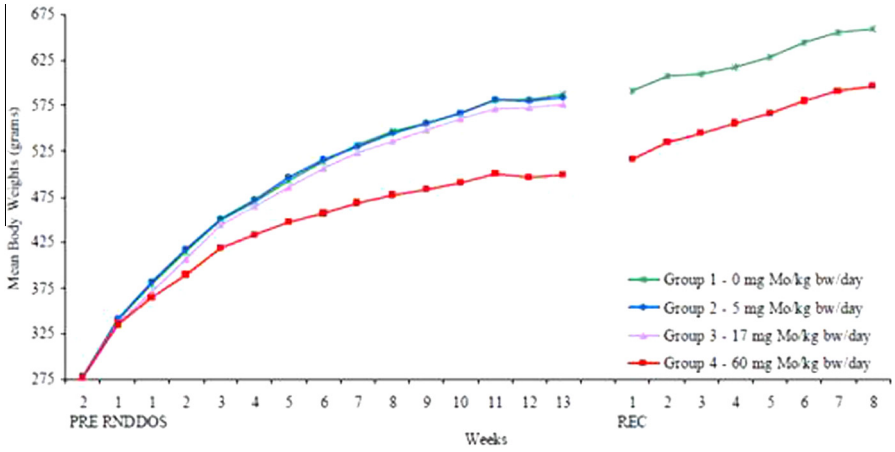

Fig. 1. Mean body weights of male rats in 90 day toxicity study of sodium molybdate dihydrate with 60 day recovery period.

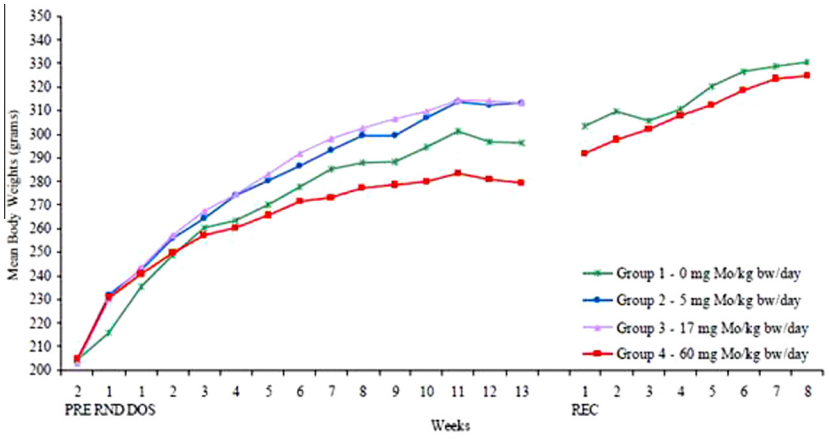

Fig. 2. Mean body weights of female rats in 90 day toxicity study of sodium molybdate with 60 day recovery period.

Table 1
Length of estrus cycles, number of cycles and number of females with irregular cycles among female rats in the 90 day toxicity study of sodium molybdate dihydrate.[a]

Dose (mg Mo/kg$_{bw}$/day)	Mean length of estrus cycle, days (No. of rats)	Mean number of cycles (No. of rats)	Number of females with irregular cycles
0	4.0 ± 0.11 (19)[b]	4.3 ± 0.7 (19)[b]	1
5	4.1 ± 0.17 (10)	3.9 ± 0.7 (10)	1
17	4.0 ± 0.26 (10)	4.1 ± 0.9 (10)	1
60	4.1 ± 0.29 (20)	4.0 ± 0.9 (20)	1

[a] Daily vaginal smears were taken from each female at approximately the same time each day and stage of estrus determined for 3 weeks starting after completing 6 weeks of treatment.
[b] Mean ± standard deviation (n).

Table 2
Mean sperm motility and counts among male rats at the terminal sacrifice in the 90 day toxicity study of sodium molybdate dihydrate (10 animals per group).

Dose (mg Mo/kg$_{bw}$/day)	Right vas deferens		Right cauda epididymis		Right testis	
	Motile sperm (%)	Progressively motile sperm (%)	Weight (g)	Sperm count (millions/g)	Weight (g)	Spermatid count (millions/g)
0 Mean ± SD	97.3	69.4	0.374	542.7	1.710	91.8
	2.6	10.9	0.036	120.5	0.080	21.5
5 Mean ± SD	97.9	60.4	0.399	640.1	1.869	91.4
	2.0	14.4	0.045	97.4	0.169	13.9
17 Mean ± SD	98.0	65.6	0.393	649.0	1.797	89.4
	1.4	8.1	0.038	152.2	0.135	18.7
60 Mean ± SD	98.1	59.0[a]	0.374	520.9	1.745	84.7
	1.3	6.8	0.032	132.1	0.132	17.1

[a] Significantly different from the control group ($p < 0.05$).

Table 3
Absolute and relative organ weights in male rats fed sodium molybdate dihydrate for 90 days.

Organ	Dose group (mg Mo/kg$_{bw}$/day)			
	0	5	17	60
Number of rats	10	10	10	10
Final body weight	566.8 ± 46.8[a]	556.4 ± 40.2	550.7 ± 45.1	482.4 ± 29.5[c]
Absolute organ weight (g, unless otherwise noted)				
Brain	2.19 ± 0.08	2.15 ± 0.12	2.10 ± 0.08[b]	2.07 ± 0.08[c]
Kidneys	3.34 ± 0.34	3.34 ± 0.29	3.23 ± 0.23	3.11 ± 0.19
Liver	14.79 ± 1.26	15.13 ± 1.41	14.91 ± 1.67	12.07 ± 0.69[c]
Heart	1.77 ± 0.25	1.65 ± 0.11	1.62 ± 0.18	1.50 ± 0.13[c]
Spleen	0.99 ± 0.20	0.91 ± 0.11	0.99 ± 0.20	0.79 ± 0.11[c]
Thymus	0.266 ± 0.088	0.283 ± 0.081	0.257 ± 0.083	0.252 ± 0.072
Thyroid/para (mg)	32.7 ± 5.9	34.0 ± 8.5	32.2 ± 6.2	31.1 ± 4.9
Adrenal glands (mg)	72.0 ± 9.3	66.5 ± 8.7	69.7 ± 11.9	69.0 ± 9.8
Pituitary gland (mg)	18.8 ± 1.9	16.1 ± 1.5[b]	16.9 ± 2.0[b]	18.1 ± 2.0[b]
Testes	3.43 ± 0.17	3.63 ± 0.40	3.60 ± 0.26	3.55 ± 0.26
Prostate w/semis	3.20 ± 0.30	3.30 ± 0.25	3.26 ± 0.38	3.09 ± 0.26
Epididymides	1.58 ± 0.05	1.64 ± 0.20	1.67 ± 0.12	1.59 ± 0.15
Relative organ weight (% body weight, unless otherwise noted)				
Brain	0.388 ± 0.025	0.388 ± 0.032	0.383 ± 0.030	0.431 ± 0.027[c]
Kidneys	0.591 ± 0.060	0.600 ± 0.043	0.588 ± 0.046	0.646 ± 0.035[b]
Liver	2.61 ± 0.16	2.72 ± 0.25	2.71 ± 0.20	2.51 ± 0.17
Heart	0.315 ± 0.052	0.296 ± 0.015	0.296 ± 0.032	0.311 ± 0.029
Spleen	0.176 ± 0.039	0.165 ± 0.027	0.180 ± 0.033	0.164 ± 0.022
Thymus	0.046 ± 0.013	0.051 ± 0.015	0.046 ± 0.014	0.052 ± 0.014
Thyroid/para (10^{-3}%)	5.8 ± 1.1	6.1 ± 1.2	5.8 ± 0.8	6.4 ± 0.8
Adrenal glands (10^{-3}%)	12.7 ± 1.6	12.0 ± 1.5	12.7 ± 2.1	14.3 ± 1.9
Pituitary gland (10^{-3}%)	3.3 ± 0.3	2.9 ± 0.2	3.1 ± 0.4	3.7 ± 0.3[c]
Testes	0.609 ± 0.047	0.654 ± 0.080	0.655 ± 0.042	0.737 ± 0.066[c]
Prostate w/semis	0.566 ± 0.052	0.594 ± 0.049	0.599 ± 0.112	0.643 ± 0.074[b]
Epididymides	0.280 ± 0.022	0.296 ± 0.045	0.305 ± 0.035	0.330 ± 0.039[c]

[a] Mean ± standard deviation.
[b] Significantly different from control group at $p < 0.05$.
[c] Significantly different from control group at $p < 0.01$.

3.6. Organ weights

In males given 60 mg Mo/kg$_{bw}$/day, absolute organ weights were statistically significantly decreased compared to control values for the brain, liver, heart, spleen and pituitary, presumably due to reduced body weight since no significant decrease in any organ weight relative to body weight was observed (Table 3). A small, but statistically significant, decrease in absolute brain weight was also seen in males at 17 mg Mo/kg$_{bw}$/day. Brain weight is typically conserved when body weight decreases. However, the decreases in absolute brain weight were not considered biologically significant, because the percent reduction in brain weight (5% at the high dose) was only one-third of the percent reduction in body weight (15% at the high dose), there was no evidence of any histopathological effects in the brain, and a similar effect was not observed in females. Statistically significant decreases in absolute pituitary weight were also observed in males at 5 and 17 mg Mo/kg$_{bw}$/day but were considered spurious based on the small magnitude of the effect, the absence of dose–response, and a lack of correlative histopathologic findings.

In females, which exhibited less evidence of reduced body weight, there were no significant effects on organ weights at any dose level, with the exception of the thyroid (Table 4).

Females given 5, 17 or 60 mg Mo/kg$_{bw}$/day had small but statistically significant decreases in absolute and relative thyroid weights; however, these differences were not considered treatment-related since the magnitude of the decreases were small and not dose-related, there was no histopathological correlate, a similar effect was not observed in males, and the concurrent control value was high compared to historical controls.

3.7. Pathology

There were no macroscopic findings at necropsy related to administration of the test material. Microscopic findings considered possibly to be related to the test substance were limited to changes in the kidneys in females at 60 mg Mo/kg$_{bw}$/day. Two females from the 60 mg Mo/kg$_{bw}$/day dose group showed slight diffuse hyperplasia of the proximal tubules in the kidney. Although this finding was present in only two female rats, it is uncommon as a background finding in this age of animal, and therefore it was presumed to be substance-related. Following the 60-day recovery phase, proximal tubule hyperplasia in the kidneys of females administered 60 mg Mo/kg$_{bw}$/day was not observed in any of the animals.

In males, there were increased incidences of 'minimal' and 'slight' vacuolation in the cells of the zona fasiculata in the adrenal cortex of males given 5 or 60 mg Mo/kg$_{bw}$/day. A dose–response relationship was not apparent, and this finding was considered incidental and unrelated to test substance administration. Increased cortical vacuolation of the adrenal is a relatively common background finding in rats that generally reflects normal but variable physiological activity. There were no test substance-related changes in the male or female reproductive tissues.

3.8. Serum, blood and tissue concentrations of molybdenum and other elements

Dietary administration of sodium molybdate dihydrate produced a dose-related increase in molybdenum concentrations in the serum and whole blood (Table 5). The males had higher serum and/or whole blood levels of molybdenum than the females (~23% on average for the three treated groups) at all dose levels at both

Table 4
Absolute and relative organ weights in female rats fed sodium molybdate dihydrate for 90 days.

Organ	Dose group (mg Mo/kg$_{bw}$/day)			
	0	5	17	60
Number of rats	10	10	10	10
Final body weight	282.9 ± 13.2[a]	298.3 ± 31.3	298.6 ± 31.0	266.9 ± 22.5
Absolute organ weight (g, unless otherwise noted)				
Brain	1.94 ± 0.08	1.92 ± 0.09	1.94 ± 0.08	1.90 ± 0.08
Kidneys	1.88 ± 0.17	1.96 ± 0.27	1.90 ± 0.17	1.83 ± 0.18
Liver	7.62 ± 0.90	7.90 ± 1.18	7.67 ± 0.70	7.07 ± 0.67
Heart	1.06 ± 0.06	1.11 ± 0.16	1.08 ± 0.11	1.00 ± 0.12
Spleen	0.54 ± 0.07	0.54 ± 0.09	0.53 ± 0.06	0.48 ± 0.09
Thymus	0.269 ± 0.071	0.304 ± 0.065	0.252 ± 0.069	0.247 ± 0.073
Thyroid/para (mg)	28.5 ± 4.4	25.0 ± 3.0[b]	24.9 ± 3.0[b]	25.1 ± 4.1[b]
Adrenal glands (mg)	66.6 ± 13.6	65.4 ± 15.7	67.7 ± 12.4	61.1 ± 8.5
Pituitary gland (mg)	22.1 ± 2.8	24.9 ± 4.9	21.4 ± 5.3	21.5 ± 3.5
Ovaries	0.087 ± 0.018	0.075 ± 0.014	0.082 ± 0.013	0.077 ± 0.017
Uterus w/ cervix	0.887 ± 0.454	0.697 ± 0.142	0.739 ± 0.180	0.830 ± 0.195
Relative organ weight (% body weight, unless otherwise noted)				
Brain	0.687 ± 0.039	0.650 ± 0.062	0.657 ± 0.086	0.716 ± 0.053
Kidneys	0.666 ± 0.046	0.658 ± 0.060	0.640 ± 0.074	0.686 ± 0.062
Liver	2.69 ± 0.28	2.64 ± 0.20	2.58 ± 0.16	2.65 ± 0.11
Heart	0.375 ± 0.020	0.371 ± 0.031	0.362 ± 0.030	0.374 ± 0.023
Spleen	0.192 ± 0.024	0.181 ± 0.031	0.177 ± 0.013	0.179 ± 0.028
Thymus	0.095 ± 0.024	0.101 ± 0.015	0.084 ± 0.020	0.093 ± 0.026
Thyroid/para (10^{-3}%)	10.1 ± 1.6	8.5 ± 1.2[b]	8.4 ± 1.0[b]	9.4 ± 1.6[b]
Adrenal glands (10^{-3}%)	23.6 ± 5.1	22.0 ± 5.1	23.1 ± 5.9	22.9 ± 2.4
Pituitary gland (10^{-3}%)	7.8 ± 1.0	8.3 ± 1.1	7.3 ± 2.0	8.0 ± 0.9
Ovaries (10^{-3}%)	30.8 ± 6.2	25.5 ± 5.8	27.7 ± 5.9	29.2 ± 7.5
Uterus w/ cervix	0.315 ± 0.166	0.236 ± 0.053	0.249 ± 0.058	0.312 ± 0.075

[a] Mean ± standard deviation .
[b] Significantly different from control group at $p < 0.05$.

weeks 4 and 12 of treatment (Table 5). This difference is not explained by the test substance intake results that showed the females had higher actual intake levels (0, 5.4, 19.0, and 65.2 mg Mo/kg$_{bw}$/day) compared to males (0, 4.5, 15.1, and 54.8 mg Mo/kg$_{bw}$/day) at the nominal dose levels of 0, 5, 17, and 60 mg Mo/kg$_{bw}$/day.

There was no significant accumulation of molybdenum in the serum, since the week 12 serum results were only slightly greater than week 4 serum results. Compared to serum levels, the whole blood levels in week 12 were consistently lower (~55% on average for the three treated groups, both sexes) suggesting that the majority of the molybdenum was in the plasma rather than in the red blood cells. During the recovery period, there was a rapid and substantial reduction in serum molybdenum concentrations at Days 2 and 7 after cessation of treatment in the group given 60 mg Mo/kg$_{bw}$/day.

Dose-related increases in liver and kidney concentrations of molybdenum (dry weight) were observed at the termination of exposure (Table 5). In contrast to the results in serum and blood, the concentrations of molybdenum in the liver and kidney were higher in females than males at all dose levels. Sixty days after exposure, nearly complete recovery to control molybdenum concentrations was observed in the liver, but not in the kidney.

Consistent with the well-known interaction between molybdenum and copper levels in the body, statistically significant increases in copper concentrations in the serum, whole blood, liver and kidney were observed in both males and females given 60 mg Mo/kg$_{bw}$/day (Table 6). In the middle dose group (17 mg Mo/kg$_{bw}$/day), the levels of copper in the serum in males, and serum, liver and kidney in females were also significantly increased, although to a lesser extent than observed at the high dose. At the low dose (5 mg Mo/kg$_{bw}$/day), the only statistically signifi-

Table 5
Molybdenum concentrations (mean ± standard deviation) in serum, whole blood, liver and kidney in rats given sodium molybdate dihydrate in the diet.

Dose (mg Mo/kg$_{bw}$/day)	Serum week 4 (ng/mL)	Serum week 12 (ng/mL)	Whole blood week 12 (ng/mL)	Serum Day 2 recovery (ng/mL)	Serum Day 7 recovery (ng/mL)	Liver Terminal sacrifice (μg/g)	Kidney Terminal sacrifice (μg/g)	Liver End of recovery (μg/g)	Kidney end of recovery (μg/g)
Male									
0[a]	18.72 ± 3.57	19.44 ± 7.18	11.97 ± 1.68	19.10 ± 4.92	20.62 ± 4.89	2.22 ± 0.19	0.93 ± 0.16	1.88 ± 0.40	0.91 ± 0.15
5	1332 ± 379.0	1309 ± 215.5	912.5 ± 212.0	N.D.	N.D.	2.53 ± 0.28	2.30 ± 0.79	N.D.	N.D.
17	4688 ± 616.9	4674 ± 1670	2930 ± 778.3	N.D.	N.D.	4.00 ± 1.23	9.52 ± 6.91	N.D.	N.D.
60	16277 ± 3660	18497 ± 4339	9903 ± 2291	4382 ± 2365	2426 ± 1187	11.49 ± 5.35	41.66 ± 31.41	2.30 ± 0.36	7.04 ± 5.93
Female									
0[a]	19.86 ± 10.65	17.93 ± 3.47	11.11 ± 2.26	15.14 ± 2.06	33.37 ± 49.15	2.46 ± 0.28	0.94 ± 0.13	2.71 ± 0.18	0.97 ± 0.16
5	991.9 ± 461.3	1121 ± 460.0	720.4 ± 329.1	N.D.	N.D.	3.51 ± 0.45	3.83 ± 2.74	N.D.	N.D.
17	3371 ± 710.4	4312 ± 985.7	2628 ± 479.0	N.D.	N.D.	4.92 ± 0.62	10.94 ± 4.36	N.D.	N.D.
60	13176 ± 3905	15532 ± 5938	7736 ± 2431	6447 ± 5431	2842 ± 2201	12.99 ± 3.65	55.04 ± 27.49	4.58 ± 2.29	16.86 ± 18.29

N.D. = not determined.
[a] Group 1 animals received approximately 0.08 mg Mo/kg$_{bw}$/day from the diet.

Table 6
Copper concentrations (mean ± standard deviation) in serum, whole blood, liver and kidney in rats given sodium molybdate dihydrate in the diet.

Dose (mg Mo/kg$_{bw}$/day)	Serum week 4 (ng/mL)	Serum week 12 (ng/mL)	Whole blood week 12 (ng/mL)	Serum Day 2 recovery (ng/mL)	Serum Day 7 recovery (ng/mL)	Liver (μg/g) terminal sacrifice	Kidney (μg/g) terminal Sacrifice	Liver (μg/g) end of recovery	Kidney (μg/g) end of recovery
Male									
0	1.26 ± 0.29	1.43 ± 0.30	1.12 ± 0.15	1.91 ± 1.22	1.49 ± 0.21	16.96 ± 1.93	30.19 ± 10.59	14.47 ± 2.64	26.31 ± 10.36
5	1.47 ± 0.18	1.62 ± 0.27	1.12 ± 0.14	N.D.	N.D.	19.47 ± 2.97	31.13 ± 6.28	N.D.	N.D.
17	1.74 ± 0.39[b]	2.10 ± 0.68[a]	1.25 ± 0.28	N.D.	N.D.	18.77 ± 3.00	39.70 ± 13.67	N.D.	N.D.
60	4.51 ± 2.21[b]	5.79 ± 2.91[b]	2.53 ± 1.21[b]	4.14 ± 1.44[b]	2.92 ± 0.69[b]	26.32 ± 7.55[a]	86.04 ± 52.93[a]	15.14 ± 1.25	43.39 ± 16.77[a]
Female									
0	1.77 ± 0.21	1.95 ± 0.23	1.32 ± 0.15	2.03 ± 0.48	1.91 ± 0.19	19.21 ± 2.06	43.54 ± 11.39	19.16 ± 1.78	43.84 ± 19.83
5	1.97 ± 0.26	2.06 ± 0.34	1.34 ± 0.23	N.D.	N.D.	23.85 ± 3.62[a]	64.34 ± 24.17	N.D.	N.D.
17	2.12 ± 0.47	2.56 ± 0.72[a]	1.40 ± 0.24	N.D.	N.D.	25.16 ± 5.06[a]	67.06 ± 23.77[a]	N.D.	N.D.
60	4.51 ± 2.17[b]	6.63 ± 4.36[b]	2.14 ± 0.50[b]	7.02 ± 4.16[b]	3.56 ± 1.50[b]	36.32 ± 7.00[b]	138.72 ± 56.34[b]	22.34 ± 4.87	74.33 ± 40.12

N.D. = not determined.
[a] Difference from relative control group statistically significant $p < 0.05$.
[b] Difference from relative control group statistically significant $p < 0.001$.

cant difference was an increase in the copper concentration in the liver of females.

4. Discussion

This study is the first published 90-day study of ingested molybdenum in animals performed according to current guidelines (OECD TG 408) and under GLP conditions. The study results did not demonstrate toxicity in rats given sodium molybdate dihydrate in the diet at dose levels of 5 and 17 mg Mo/kg/day for 90 days. However, at the highest dose level (60 mg Mo/kg$_{bw}$/day), toxicity was evident in both males and females. This high dose is at least 20,000-fold higher than the current estimates of human intake of 1–2 μg Mo/kg$_{bw}$/day derived from molybdenum-containing food and water (Turnlund and Friberg, 2007).

At the end of the 90-day exposure period, the high dose males and females weighed 15.1% and 5.6% less, respectively, than controls. At the high dose, the decreased growth observed in males was associated with decreased food consumption, and there was evidence of reduced food conversion efficiency in both males and females. In earlier toxicity studies in rats, exposure to molybdenum also produced a decreased body weight gain; however, pair-feeding experiments demonstrated that reduced food intake was not the primary cause of weight loss produced by exposure to molybdenum (Arrington et al., 1965). The greater effect on the growth of males compared to females at the high dose is not due to a difference in the ingested dose of Mo since the actual mean dose of Mo ingested by high-dose males and females was approximately 55 and 65 mg Mo/kg$_{bw}$/day, respectively. However, the greater effect on the growth of males may be related to the fact that serum levels of Mo at the high dose were slightly higher (about 23% on average) among males compared to females.

Histopathological evaluation revealed test substance-related findings in the kidneys (slight diffuse hyperplasia of the proximal tubules) of two 60 mg Mo/kg$_{bw}$/day females at the terminal sacrifice, which were not present in females sacrificed at the end of the recovery period of 60 days. No other microscopic effects were observed that appeared to be treatment-related. The effect of molybdenum on renal function was previously studied in male rats given 40 or 80 mg Mo/kg$_{bw}$/day by gavage for 8 weeks (Bompart et al., 1990). In that gavage study, nephrotoxicity and decreased body weight were observed at 80 mg Mo/kg/day, but not at 40 mg Mo/kg$_{bw}$/day. It is interesting to note that excretion of molybdenum occurs predominantly via the kidneys, which exert homeostatic regulation over molybdenum balance (CDC, 2012). Whether the elevated concentrations of copper observed in the kidneys of the high dose females in the present study (138.7 μg/g vs. 43.5 μg/g in controls) may have played some role in the histopathological changes in the kidneys is not known, although the kidney is a target organ for copper toxicity (Hebert, 1993).

The results of the current study do not confirm earlier reports of reproductive effects on male and female rats exposed to molybdenum. Pandey and Singh (2002) reported adverse effects of sodium molybdate on sperm count, motility and morphology in Druckery rats administered sodium molybdate by gavage at dose levels of 12 and 20 mg Mo/kg$_{bw}$/day five days per week for a 60 day period. In contrast, no adverse treatment-related effects on the testes (weight or histopathology), sperm counts, motility or morphology were seen in the current study in rats administered larger oral doses (up to 60 mg Mo/kg$_{bw}$/day) for a longer duration (90 days). Possible factors contributing to the discrepancy in findings between the two studies include the different strains of rats and the different routes of administration (gavage vs. diet), although no significant difference in toxicity was observed between the gavage and dietary routes of exposure in our 28-day range-finder study. However, the Pandey and Singh (2002) study was poorly reported with no clear description of the effects on the testes or the actual numbers of animals affected, and there were apparent inconsistencies in the statistical analyses.

The results of the current study are also inconsistent with those of Fungwe et al. (1990), who reported a significantly prolonged length of the estrus cycle in rats exposed to molybdenum in the drinking water for 6 weeks (Fungwe et al., 1990). Although Fungwe et al. (1990) did not describe the dose levels in terms other than the concentration of molybdenum in the drinking water, others have estimated that the NOAEL and LOAEL for estrus cycle effects were 0.9 and 1.6 mg Mo/kg$_{bw}$/day, respectively, in this study. In contrast, the results of the present study showed no effect on the estrus cycles in rats given molybdenum in the diet for 90 days at dose levels up to 60 mg Mo/kg$_{bw}$/day. The group size was larger in the current study (n = 10–20) than in the Fungwe et al. (1990) study (n = 6). Both studies used Sprague–Dawley rats, and it is unlikely that the difference in the routes of exposure (diet vs. drinking water) would explain such different findings. Other studies in animals have demonstrated that the effects of molybdenum on growth are more pronounced under situations where dietary copper intake is low (EPA, 2003). The dietary concentration of copper was determined to be adequate in the present study, but the dietary concentration of copper was not described in the Fungwe et al. (1990) study. The inability of the present study to confirm the effects of molybdenum on the estrus cycle reported by Fungwe et al. (1990) is important because that study has been used as the critical study in risk assessments of molybdenum (Vyskocil and Viau, 1999; IOM, 2001; EPA, 2003).

Molybdenum was clearly absorbed from the diet in the present study, as evidenced by the substantial dose-related increases of molybdenum in the serum, blood, liver and kidney. For example, in the high dose males at the termination of the 90-day exposure period, the molybdenum concentrations in serum, blood, liver and kidney were approximately 950, 825, 5.2 and 45 times greater than the respective control values. Neither Fungwe et al. (1990) nor Pandey and Singh (2002) measured the concentrations of molybdenum in serum, blood or tissues, precluding direct comparisons with the molybdenum levels observed in the present study.

No evidence of a treatment-related effect on the reproductive organs or secondary reproductive organs was reported in a 90-day and a 2-year repeat-dose toxicity study of inhaled molybdenum trioxide, a less soluble molybdenum substance than is sodium molybdate dihydrate, in both mice and a different strain of rats (NTP, 1997). Although the test substance, strain of rats, and exposure route used by NTP differed from that employed in the present study, the substance circulating in the blood (i.e., molybdate ion) would be the same for both substances (Mitchell, 2009). Thus, systemic exposure to molybdenum may be compared based on blood (or serum) concentrations of molybdenum. The mean blood molybdenum concentration in male rats at the highest exposure concentration (100 mg/m^3 molybdenum trioxide) in the NTP 2-year inhalation study of molybdenum trioxide was about 60% of the mean blood molybdenum concentration observed among high dose males in the current 90-day dietary study, showing substantial absorption of molybdenum in the NTP study and with no adverse effects observed on the reproductive organs.

In the current 90-day toxicity study, ingestion of sodium molybdate dihydrate produced dose-related increases in copper levels in serum, blood, kidneys and liver. For example, at the end of the 90-day exposure period, serum copper levels among high dose males and females were 405% and 340%, respectively, of the control values. Previous toxicity studies of molybdenum in rodents have reported that high doses of molybdenum produced toxicity, which was associated with very high blood and tissue levels of copper. The copper, however, was tightly bound to carrier proteins in the blood and tissues, and it was suggested that, despite the elevated copper levels, the toxicity of molybdenum was actually related to a reduction in bioavailable copper (Compere et al., 1965; Nederbragt, 1980; Nederbragt and van den Hamer, 1981; Nederbragt, 1982). Measuring the speciation, binding and excretion of copper was beyond the scope of the present study. The changes in copper levels at 5 and 17 mg Mo/kg_{bw}/day were considered to represent a physiological adaptation, not a toxic response, since symptoms of toxicity attributable to the test material were not observed and the tissue levels, even at the highest dose level were well below those associated with toxicity in other studies (Hebert, 1993; Stern, 2010).

5. Conclusion

In conclusion, dietary administration of sodium molybdate dihydrate providing a dose of 60 mg Mo/kg_{bw}/day for 90 days clearly affected body weight gain in male and female rats, and was associated with mild renal effects in females. Elevated levels of molybdenum and copper were found in the serum, blood, liver and kidneys of rats treated with 60 mg Mo/kg_{bw}/day. Lower doses of 5 and 17 mg Mo/kg_{bw}/day did not cause any treatment-related toxicity. No treatment-related adverse effects on the reproductive organ weights or histopathology, estrus cycles or sperm parameters were observed at any dose level. Thus, the NOAEL for molybdenum in male and female rats was determined to be 17 mg Mo/kg_{bw}/day based on effects on reduced body weight gain in males and females, and renal effects in two females at 60 mg Mo/kg_{bw}/day. The NOAEL for gonadal, sperm and estrus cycle effects was 60 mg Mo/kg_{bw}/day.

Conflicts of interest

FMS and FJM are consultants to the International Molybdenum Association (IMOA). SC is an employee of IMOA. AKT works in the molybdenum industry.

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