Reproductive Toxicology 49 (2014) 202–208

Contents lists available at ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

Developmental toxicity study of sodium molybdate dihydrate administered in the diet to Sprague Dawley rats

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ARTICLE INFO

Article history:
Received 8 June 2014
Received in revised form 13 August 2014
Accepted 4 September 2014
Available online 16 September 2014

Keywords:
Molybdenum
Molybdate
Diet
Developmental
Toxicity
Fetus
Copper
Rats

ABSTRACT

Molybdenum is an essential nutrient for humans and animals and is a constituent of several important oxidase enzymes. It is normally absorbed from the diet and to a lesser extent from drinking water and the typical human intake is around 2 μg/kg bodyweight per day. No developmental toxicity studies to contemporary standards have been published and regulatory decisions have been based primarily on older studies where the nature of the test material, or the actual dose levels consumed is uncertain.

In the current study the developmental toxicity of sodium molybdate dihydrate as a representative of a broad class of soluble molybdenum(VI) compounds, was given in the diet to Sprague Dawley rats in accordance with OECD Test Guideline 414. Dose levels of 0, 3, 10, 20 and 40 mg Mo/kg bw/day were administered from GD6 to GD20. No adverse effects were observed at any dose level on the dams, or on embryofetal survival, fetal bodyweight, or development, with no increase in malformations or variations. Significant increases in serum and tissue copper levels were observed but no toxicity related to these was observed. The NOAEL observed in this study was 40 mg Mo/kg bw/day, the highest dose tested.

1. Introduction

Molybdenum (Mo) is an essential nutrient for humans, as well as for animals and plants [1]. In humans and other mammals, molybdenum is a key component of several important enzymes, including aldehyde oxidase, sulfite oxidase, and xanthine oxidase [2]. Human exposure to molybdenum may occur via the diet, drinking water, or inhalation from occupational exposure from mining operations and various industrial uses. The USA National Research Council (NRC) has published a Recommended Dietary Allowance (RDA) of molybdenum for adult men and women of 45 μg/day. The average dietary intake of molybdenum by adult men and women is stated to be 109 and 76 μg/day, respectively, and a Tolerable Upper Intake Level (UL) is set at 2 mg/day [1]. In a review of intakes in the USA and Europe, an average adult human intake of molybdenum of about 2 μg/kg bodyweight per day has been reported [3], corresponding to about 140 μg/day for a 70 kg person.

In humans, the toxicity of molybdenum compounds has been observed to be relatively low. In general, soluble molybdenum(VI) compounds (e.g., sodium molybdate dihydrate) are more toxic than insoluble molybdenum compounds [4]. Dietary intake of high levels of molybdenum can alter copper metabolism in humans, resulting in an increase in copper excretion and elevated levels of bound plasma copper [5]. The symptoms of molybdenum toxicity in animals are similar to those of copper deficiency, and copper supplementation is effectively used to reverse molybdenum toxicity (and vice versa).

Published toxicity studies in rats have suggested that oral administration of sodium molybdate dihydrate at daily dose levels of 30 and 50 mg/kg bw/day of sodium molybdate can induce testicular damage [6] and, at drinking water concentrations of 10 mg Mo/L or greater, interferes with oestrus cycles [7]. The actual dose levels (mg Mo/kg/day) used by Fungwe et al. [7] study are unclear because the authors reported only drinking water concentrations (0, 5, 10, 50 and 100 mg Mo/L) without data on maternal body weights and drinking water consumption; however, by making assumptions, Vyskocil and Viau [4] estimated the dose levels in the Fungwe et al. [7] study were approximately 0, 0.9, 1.6, 8.3 and 16.7 mg Mo/kg/day, respectively. These estimates, while tenuous, will be used throughout this article. Importantly, no effect

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http://dx.doi.org/10.1016/j.reprotox.2014.09.001

on semen quality, oestrus cycles or the histopathology of male or female reproductive organs was observed in a recent 90-day, OECD Test Guideline 408 compliant, toxicity study in rats fed diets containing sodium molybdate dihydrate at higher dose levels up to 60 mg Mo/kg bw/day [8].

A review of the literature revealed limited studies of the potential developmental toxicity of molybdenum compounds. Fungwe et al. [7] reported increased resorptions and decreased fetal body weight among the offspring of pregnant Sprague Dawley rats given approximately 1.6 mg Mo/kg bw/day as sodium molybdate dihydrate in the drinking water from postnatal day 21 (PND 21) through development and mating to gestation day 21 (GD 21). External examination of the fetuses did not reveal any significant increase in the incidence of congenital abnormalities. Histopathological findings of fetuses of dams given approximately 1.6 mg Mo/kg bw/day or greater were reported as "fetuses appeared to be at an earlier stage of embryonic development," i.e., a finding consistent with reduced fetal body weight. An important criticism of the study however, is that it is not possible to determine the exact dose levels of molybdenum consumed by the pregnant dams as no data on water consumption were reported. This is further reviewed in the Section 4.

In another developmental toxicity study, pregnant Wistar rats were given 0, 10, 20, or 40 mg/kg bw/day of a substance described as "molybdenum acid ammonium salts" by gavage on GD 7–16, but the specific chemical identity, purity or source of the test material was not provided [9]. Significant decreases in the percentage of live fetuses per litter, fetal bodyweight, and statistically significant increases in the incidences of external, visceral and skeletal malformations were reported at 40 mg/kg bw/day. However the study is very poorly reported, with obvious errors. For example, it is stated that 75 pregnant rats were randomly divided into 5 groups of 15 rats with a control and 4 treatment groups. In the control group, the mean number of implanted embryos is reported as 14.11 ± 2.34, yet the total live and dead fetuses are reported as 103 and 7 respectively, with a 93.6% rate of live fetuses, which is clearly incorrect since a total of over 200 implants would be expected. Similar numbers are reported in the other treated groups. It is therefore impossible to assess the results of this study.

Given the uncertainties of the developmental toxicity studies in the literature, the objective of the current study was to assess the potential developmental toxicity of sodium molybdate dihydrate when given in the diet to Sprague Dawley rats in a GLP-compliant guideline study. This study was conducted at RTI International, USA in accordance with OECD Test Guideline 414 [10]. Sodium molybdate dihydrate was selected as a source of the molybdate ion $[MoO_4]^{2-}$ that would be representative of the broader class of soluble molybdenum(VI) compounds, since at physiological pH in biological systems, dissolved molybdenum(VI) compounds exist in the form of molybdate ion.

2. Materials and methods

2.1. Test article and diet analysis

Sodium molybdate dihydrate ($Na_2MoO_4 \cdot 2H_2O$; SMD), white crystals, with a purity of 99.9% was supplied by Climax Molybdenum, Phoenix, AZ, USA. For administration to the animals in the diet, the SMD was formulated in Certified Purina Rodent Chow 5002 (Purina Mills, Inc., Richmond, IN, USA) at RTI International at various concentrations using GLP-compliant procedures and equipment. Analyses of test chemical in the dosed feed formulations were conducted by Justin Zyskowski, DCPAH, at Michigan State University, Lansing, MI, USA. Homogeneity of the dosed feed formulations was evaluated at the lowest and highest proposed dietary concentrations.

2.2. Animals and treatment

One hundred and twenty five mated Sprague Dawley rats, (nomenclature Crl:CD(SD)), were obtained from Charles River Laboratories, Raleigh, NC, at 8–10 weeks of age upon arrival on gestational day (GD) 0 or 1. Date of detecting a positive vaginal smear at the vendor was designated GD 0. All animals were used in compliance with the NRC Guide for the use of animals [11]. The test animals were divided into 5 groups of 25 by stratified randomization by body weight on GD 3, to provide uniform mean body weights across dose groups (±20%). They were housed in the RTI Animal Research Facility (ARF) in non-barrier rooms in solid-bottom, polycarbonate caging, singly housed during gestation to GD 20, with room temperature 72 ± 3 °F (~22 ± 2 °C); RH 50 ± 20%; light cycle 12-h light:12-h dark; and 10–15 air changes per hour. The Purina diet and City of Durham tap water were available ad libitum. The drinking water was analyzed monthly by the City of Durham, NC.

2.3. Elemental analysis of serum, placentae, livers and kidneys

At the termination of the study, blood and other tissue samples were taken from 10 rats per group as described in Section 2.6.1 and analyzed for molybdenum, copper, zinc, manganese, iron, cobalt and selenium. Collected samples were frozen in liquid nitrogen and shipped on dry ice to the analytical laboratory for analysis 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.

2.4. Study design

2.4.1. Treatment groups

Timed-mated females were assigned to treatment groups by stratified randomization by body weight as described above. This study was conducted with 4 treatment groups and a vehicle control group, each comprising 25 timed-mated females.

Concentrations of sodium molybdate dihydrate (SMD) in the diet in parts per million (ppm; mg/kg feed) were prepared, based on measured intakes from a range-finding study [12], to provide target molybdenum intakes of 0, 3, 10, 20 and 40 mg Mo/kg bw/day. The corresponding sodium molybdate dihydrate concentrations in the diet were 0, 100, 338, 675 and 1350 ppm, respectively. On GD 0 or 1 (day of arrival at RTI) to GD 5, dams received the control feed ad libitum. From GD 6 through GD 20, the dosed feed was presented to the dams in feed jars, available ad libitum 7 days/week.

2.5. Maternal observations

Clinical observations (out of cage) were conducted and recorded at least once daily. Clinical observation records included the time the clinical signs were observed and the severity and duration of the signs. Daily morbidity/mortality checks were done twice per day, at least 6 h apart, beginning the day after receipt. Individual body weights and food consumption were measured on GD 3, 6, 9, 12, 15, 18, and 20, at approximately the same time in the morning each day.

2.6. Scheduled necropsy

2.6.1. Maternal

On GD 20 final body weights in grams were taken and all dams were euthanized by CO_2 asphyxiation. Serum from 10 arbitrarily selected females per group, collected via cardiac puncture, livers and kidneys (2 per female) and placentae from the same 10

females/group were frozen in liquid nitrogen and stored at −80 °C until shipped to the analytical laboratory, at Michigan State University, and analyzed for molybdenum, manganese, iron, cobalt, copper, zinc and selenium concentrations.

Uterus, liver and kidney weights were recorded, ovarian corpora lutea counts performed, and maternal gross lesions were retained in 10% formalin. Histopathological evaluation of livers and kidneys from the remaining 15 pregnant control females and from 15 pregnant high dose females were performed.

2.6.2. Embryofetal evaluation

Uterine implantation sites were classified as live fetus, dead fetus, early resorption or late resorption. Live fetuses were dissected from the uterus after maternal termination on GD 20 and immediately placed on a moist paper towel over a tray of ice, a procedure that induces anesthesia and subsequent death by lowering the core body temperature below 25 °C [13,14]. All live fetuses were weighed and sexed and subjected to external examination. Half of the fetuses were then evaluated by fresh visceral examination after removal and fixation of the heads in Bouin's fluid, and all fetuses, intact and decapitated, were stained in Alizarin Red S (for bone) and Alcian Blue (for cartilage) [15]. The intact fetal skeletons (50%) were then evaluated.

2.7. Statistical analyses

All RTI-generated data were collected on the validated RTI Instem Provantis™ System, and all statistical analyses were performed using Provantis™ software. For all statistical tests, $P < 0.05$ was used as the criterion for significance and the dam or litter was used as the statistical unit as appropriate. Quantitative continuous data (e.g., maternal body weights and weight gains, feed consumption in g/day and g/kg body weight/day) were subjected to the Provantis™ generalized ANOVA/ANCOVA test. This decision tree includes analysis of variance (ANOVA) and covariance (ANCOVA), nonparametric analysis of variance, pairwise tests [16,17] for parametric and nonparametric data, and Levene's test [18] for homogeneity of variance. For each variable analyzed, where there was evidence of differences between groups, the methodology also identified those groups, which differed from the control group for these variables.

The uterine weight and uterine implant data were subjected to the Kruskal–Wallis nonparametric analysis of variance, the default technique in Provantis™ [19,20]. When there was evidence of a significant group effect, pairwise comparisons of each treated group with the control group were performed using Dunnett's test on the ranks. The fetal weights and sex ratios were subjected to a 1-way mixed ANOVA, the default technique in Provantis™. When there was evidence of a significant group effect, pairwise comparisons of each treated group with the control group were performed using Dunnett's test on group least square means.

Statistical analyses of the Michigan State University analytical data on blood and tissue minerals were performed using Students *t*-test, 2 tailed, heteroskedastic compared with corresponding controls, and $P < 0.01$ was used as the criterion for significance.

3. Results

3.1. Chemical analyses of feed

Analyses indicated that the high and low dose formulations were stable for at least 28 days at room temperature and that the dietary dose formulations of sodium molybdate dihydrate (SMD) in the feed at 0, 100, 338, 675, and 1350 ppm (parts per million) were accurate; all dietary samples were well within ±10% of the target concentrations of molybdenum, and were homogeneous. The concentrations of SMD added to the diet of 0, 100, 338, 675, and 1350 ppm provided target molybdenum concentrations of 0, 40, 134, 268, and 536 ppm. The analyses of molybdenum in the feed samples gave actual concentrations of molybdenum of 1.75–1.80, 39.7–40.8, 134–139, 264–268, and 540–542 ppm for the 0, 40, 134, 268, and 536 ppm feeds, respectively.

3.2. Maternal results

Of the 125 female rats in the study, only one, in the 20 mg Mo/kg bw/day dose group, was not pregnant. None of the females died during the study, and none of the pregnant females aborted or had complete resorptions at scheduled necropsy. All 25 pregnant females at 0, 3, 10, and 40 mg Mo/kg bw/day and all 24 pregnant females at 20 mg Mo/kg bw/day had live litters at scheduled termination.

Mean maternal body weights during gestation were equivalent across all groups on GD 3, 6, 9, 12, 15, 18, and 20, with no significant differences in bodyweights or bodyweight gains during treatment. The mean (±SEM) bodyweights of the groups at start of treatment on GD 6 ranged from 250 ± 2.53 g to 254 ± 2.61 g, and on GD 20 ranged from 367 ± 4.75 g to 377 ± 4.65 g with no dose related trend. The mean maternal feed consumption values in g/day and g/kg bw/day during gestation were also equivalent across all groups for all gestational intervals. The mean (±SEM) daily food intakes between GD 6 and GD 20 in the control, 3, 10, 20 and 40 mg Mo/kg bw/day groups were 21.2 ± 0.5, 22.0 ± 0.6, 22.7 ± 0.6, 22.8 ± 0.8 and 21.0 ± 0.6 g/day, respectively.

The actual mean ± SEM molybdenum intake during GD 6–20 in the nominal target dose level groups of 3, 10, 20, and 40 mg Mo/kg bw/day was 2.8 ± 0.07; 9.8 ± 0.24; 20.0 ± 0.68; and 37.5 ± 1.0 mg Mo/kg bw/day, respectively. The molybdenum content of the control diet was 1.8 mg Mo/kg diet, and the drinking water contained 0.2 μg Mo/L, giving an additional intake of 0.13 mg Mo/kg bw/day in all groups, including controls, and was not included in the above intake values.

There were no treatment- or dose-related maternal clinical signs in any female in any group. At scheduled necropsy on GD 20, there were no differences across groups for mean terminal body weight, gravid uterine weight, net body weight (terminal body weight minus gravid uterine weight), or for corrected mean body weight change from GD 3 to GD 20. Maternal terminal body weights, and absolute and relative (to terminal body weight) maternal liver and paired kidney weights were all statistically and biologically equivalent across all groups.

At terminal necropsy, all maternal kidneys were subjected to gross pathological examination from all females on study; one female at 40 mg Mo/kg bw/day exhibited kidney discoloration, pale, bilateral, misshapen kidneys, bilateral, and dilation, bilateral, of the renal pelvis, and one female at 40 mg Mo/kg bw/day exhibited right renal pelvis dilation (they both had live litters). All maternal livers were also subjected to gross pathological evaluation, with no visible (gross) lesions observed.

Histopathological evaluation of maternal livers and kidneys of the 15 (of 25) dams per group not used for elemental analyses, indicated no treatment- or dose-related incidences or severities of any histopathological findings in the top dose group animals compared with the controls.

3.3. Embryofetal results

The maternal ovarian and uterine implantation data, fetal numbers and bodyweights are shown in Table 1 and indicated no treatment- or dose-related differences across groups for the numbers of ovarian corpora lutea/female, the numbers of uterine implantations/female, for pre- or post-implantation loss, litter size

Table 1
Summary of uterine implantation data (GD20) from rats exposed to dietary sodium molybdate dihydrate (SMD) on days 6–20 of gestation (GD 6–20).

Dose group: SMD ppm in diet (mg Mo/kg bw/day)		0 0	100 3	338 10	675 20	1350 40
Number of females with live fetuses at scheduled termination		25	25	25	24	25
Total number of corpora lutea		360	364	354	345	364
Number of corpora lutea per female	Mean	14.4	14.6	14.2	14.4	14.6
	SEM	0.5	0.6	0.4	0.4	0.5
Total number of implantations		317	322	327	319	313
Number of implantations per female	Mean	12.7	12.9	13.1	13.3	12.5
	SEM	0.5	0.3	0.3	0.4	0.4
Total number of pre-implantation losses		43	42	27	26	51
Mean % per group		11.9	11.5	7.6	7.5	14.0
Total number of post-implantation losses		9	10	13	14	6
Mean % per group		2.8	3.1	4.0	4.4	1.9
Number of live fetuses per female	Mean	12.3	12.5	12.6	12.7	12.3
	SEM	0.5	0.3	0.3	0.4	0.4
Number of live fetuses as % of implantations	%	97.2	96.9	96.0	95.6	98.1
Total number of live fetuses		308	312	314	305	307[a]
Number of males		152	141	162	155	149
Number of females		156	171	152	150	157
Fetal weight (sexes combined) (g)	Mean	4.02	4.04	4.02	4.02	4.04
	SEM	0.06	0.05	0.04	0.07	0.07
Fetal weight (males) (g)	Mean	4.13	4.16	4.12	4.13	4.14
	SEM	0.06	0.06	0.05	0.07	0.06
Fetal weight (females) (g)	Mean	3.90	3.95	3.91	3.91	3.95
	SEM	0.06	0.05	0.04	0.06	0.08

[a] One fetus inadvertently not sexed.

and fetal bodyweight. No female in any group had total litter loss, i.e., completely resorbed litter.

The mean numbers of live fetuses/female were similar in all groups with no statistically or biologically significant differences among groups. There were no differences among groups for % male fetuses/dam, for mean litter weights/dam, or for individual fetal body weights, with sexes combined or separately, by sex/dam (Table 1).

A summary of fetal defects is presented in Table 2, and the individual malformations observed are shown in Table 3. There were no statistically significant dose-related increases in fetal malformations or variations (Table 2). There was 1 externally malformed fetus each at 3 and 40 mg Mo/kg bw/day; there were 2 fetuses (from 2 litters) at 10 mg Mo/kg bw/day, and 6 fetuses (from 3 litters) at 20 mg Mo/kg bw/day with head malformations, and 1, 1, 4, 2 and 1 fetuses (in 1, 1, 4, 2 and 1 litters) with skeletal malformations at 0, 3, 10, 20, and 40 mg Mo/kg bw/day, respectively. No treatment related increase in fetal visceral or skeletal variations was observed. The large numbers of fetuses with skeletal variations in all groups was anticipated from the historical control rat data from previous studies in this laboratory in this rat strain. Nasal sinus, bilateral, enlarged, classified as a head malformation, was

Table 2
Occurrence of external, visceral and skeletal malformations and variations in the fetuses of rats exposed to sodium molybdate dihydrate.

Dose group (mg Mo/kg bw/day)	0	3	10	20	40
Total number of fetuses examined	308	312	314	305	307
Total number of litters examined	25	25	25	24	25
External defects					
Number of fetuses examined[a]	307	312	313	303	307
Number showing malformations	0	1	0	0	1
Number of litters affected	0	1	0	0	1
Number showing variations	0	0	0	0	0
Number of litters affected	0	0	0	0	0
Fresh visceral body-only defects					
Number of fetuses examined	150	151	151	152	152
Number showing malformations	0	0	0	0	0
Number of litters affected	0	0	0	0	0
Number showing variations	7	4	11	2	5
Number of litters affected	5	3	8	2	4
Bouin's head defects					
Number of fetuses examined	150	151	151	152	152
Number showing malformations	0	0	2	6	0
Number of litters affected	0	0	2	3	0
Number showing variations	0	0	0	2	1
Number of litters affected	0	0	0	1	1
Skeletal defects					
Number of fetuses examined	158	161	163	153	155
Number showing malformations	1	1	4	2	1
Number of litters affected	1	1	4	2	1
Number showing variations	54	69	69	65	64
Number of litters affected	18	21	22	19	22

[a] In dose groups 0, 10 and 20 mg Mo/kg bw/day, 1, 1, and 2 fetuses, respectively were not examined externally but did have visceral or skeletal examination.

Table 3
Individual incidences of fetal malformations by dose group, dam and fetus of rats exposed to sodium molybdate dihydrate.

Dose group (mg Mo/kg bw/day)	Dam and fetus numbers	Findings
0	Dam 18, F 7	Short left rib and cartilage agenesis
3	Dam 29, F 12	Umbilical hernia
	Dam 36, F 3	Thoracic vertebral centrum, bipartite ossification and bipartite cartilage
10	Dam 51, F 10	Dilated nasal sinus
	Dam 60, F 12	Dilated nasal sinus
	Dam 63, F 8	Thoracic vertebral centrum, bipartite ossification and bipartite cartilage
	Dam 67, F 3	Thoracic vertebral centrum, bipartite ossification and bipartite cartilage
	Dam 68, F 1	Thoracic vertebral centrum, bipartite ossification and bipartite cartilage
	Dam 64, F 11	Rib 13, right, cartilage agenesis
20	Dam 78, F 1, F 4, F 5	Dilated nasal sinus
	Dam 85, F 8, F 10	Dilated nasal sinus
	Dam 100, F 10	Dilated nasal sinus
	Dam 79, F 5	Thoracic vertebral centrum, bipartite ossification and bipartite cartilage
	Dam 99, F 9	Rib 13, right, cartilage agenesis
40	Dam 125, F 7	Tail short and curly; rib 7 branched; rib 11 cartilage branched, and thoracic vertebral centrum, bipartite ossification and bipartite cartilage

Table 4
Summary of blood serum and tissue levels of elements, which showed significant dose related changes at terminal sacrifice of 10 dams per group.

Dose: SMD ppm in diet (mg Mo/kg bw/day)		0 0	100 3	338 10	675 20	1350 40
Blood serum levels						
Molybdenum	Mean (μg/ml)	0.024	0.68[a]	2.43[a]	4.87[a]	10.04[a]
	SD	0.003	0.42	0.86	0.98	2.20
Copper	Mean (μg/ml)	2.02	2.09	2.50[a]	3.07[a]	3.98[a]
	SD	0.22	0.34	0.40	0.81	1.12
Placental levels						
Molybdenum	Mean (μg/g dry)	0.75	3.40	15.74[a]	26.35[a]	54.51[a]
	SD	1.53	4.02	10.2	9.12	18.06
Copper	Mean (μg/g dry)	21.3	19.1	29.2	34.2[a]	50.7[a]
	SD	2.71	5.34	11.8	11.3	21.9
Kidney levels						
Molybdenum	Mean (μg/g dry)	1.51	5.71[a]	23.0[a]	38.0[a]	87.1[a]
	SD	0.10	2.11	9.64	14.7	15.6
Copper	Mean (μg/g dry)	42.3	44.1	52.6	63.1	118.2[a]
	SD	16.1	19.1	25.4	31.5	33.3
Liver levels						
Molybdenum	Mean (μg/g dry)	2.47	3.43[a]	6.14[a]	9.71[a]	17.8[a]
	SD	0.14	0.80	1.64	2.20	2.63
Copper	Mean (μg/g dry)	12.8	13.0	14.7[a]	17.0	19.5[a]
	SD	0.68	1.94	1.53	4.62	3.46

[a] Significant difference from controls ($P < 0.01$).

Table 5
Comparison of molybdenum concentrations in placenta and liver reported by Fungwe et al. [22], with current study results.

Fungwe et al. [22]

Dose: mg Mo/L drinking water		0	5	10	50	100
Suggested dose (mg Mo/kg bw/day)[a]		0	0.9	1.6	8.3	16.7
Placental levels						
Molybdenum	Mean (μg/g dry)	0.60	1.90	4.32	28.15	45.32
	SE	1.9	0.29	0.45	8.40	9.30
Liver levels						
Molybdenum	Mean (μg/g dry)	2.38	4.50	5.59	10.66	13.15
	SE	0.11	0.21	0.37	0.16	0.14

Current study Murray et al. (2014)

Dose: mg Mo/kg bw/day		0	3	10	20	40
Placental levels						
Molybdenum	Mean (μg/g dry)	0.75	3.40	15.74	26.35	54.51
	SD	1.53	4.02	10.20	9.12	18.06
Liver levels						
Molybdenum	Mean (μg/g dry)	2.47	3.43	6.14	9.71	17.83
	SD	0.14	0.80	1.64	2.20	2.63

[a] Suggested dose levels on bodyweight basis by Vyskocil and Viau [4] (see Section 4).

observed in 2 fetuses (from different litters) at 10 mg Mo/kg bw/day and in 6 fetuses (from 3 litters) at 20 mg Mo/kg bw/day; there were no fetuses with head malformations at 0, 3, or 40 mg Mo/kg bw/day. Fetal skeletal defects were observed in regions of the sternebrae, ribs, thoracic vertebrae and lumbar vertebrae, with no treatment- or dose-related pattern of incidences or severities.

There were clearly no dose-related incidences of fetal malformations in any group. Dilated nasal sinus as described above is an uncommon fetal finding, and it was not considered treatment- or dose-related in this study.

3.4. Chemical analyses of serum and tissues

Analysis of the blood serum, placentae, liver and kidneys for molybdenum and other elements were performed on 10 dams per group selected at scheduled necropsy. Of the elements measured (i.e., molybdenum, copper, manganese, iron, cobalt, zinc and selenium), only molybdenum and copper showed clear dose-related increases in levels. A summary of these results for molybdenum and copper is presented in Table 4. Dose-related increased copper levels in serum, kidneys, livers and placentae were variously observed across all the doses, with statistically significant increases in all at the highest dose.

4. Discussion and conclusion

This study is the first published developmental toxicity study of ingested molybdenum in animals performed according to current testing guidelines (OECD Test Guideline 414) and under GLP conditions. Dietary administration of sodium molybdate dihydrate to pregnant rats on GD 6–20 had no significant adverse effect on the developing offspring at dose levels up to 40 mg Mo/kg bw/day (i.e., 1350 ppm of sodium molybdate dihydrate in the diet). No significant effects on litter size, resorptions, sex ratio, fetal body weight, or fetal malformations and variations were observed at any dose level tested.

Although no maternal or embryofetal toxicity was observed in this study, the highest dose level of 40 mg Mo/kg bw/day used in the study is approximately 20,000-fold greater than typical equivalent human dietary intake of molybdenum (ca. 2 μg Mo/kg bw/day, as reported by Turnlund and Friberg [3]). At the high dose level, the serum level of molybdenum observed in pregnant rats on GD 20 was 10,040 ng Mo/ml, which is more than 400 times greater than the control rat values and approximately 10,000-fold higher than the typical human serum level of 1 ng Mo/ml [3].

Maternal serum and placental concentrations of copper were statistically significantly increased compared to controls at doses of ≥10 and ≥20 mg Mo/kg bw/day, respectively. However, these increases in copper levels in response to increasing doses of molybdenum are considered to represent physiological adaptations, not a toxic response at these dose levels, since neither symptoms of maternal toxicity nor developmental toxicity attributable to the test material were observed. These results are consistent with the observation of elevated serum copper in both male and female rats in a 90-day toxicity study of sodium molybdate dihydrate given in the diet at dose levels of 17 and 60 mg Mo/kg bw/day, but not at 5 mg Mo/kg bw/day [8].

The results of the current study are in contrast with those of the Fungwe et al. [7] study, in which female rats were exposed to SMD in drinking water containing 0, 5, 10, 50, or 100 mg Mo/L from weaning at 3 weeks of age until mating at around 11 weeks of age, and through pregnancy to sacrifice on GD 21. Fungwe et al. [7] reported increased resorptions and decreased fetal body weight among the offspring of pregnant rats given SMD in the drinking water, (estimated doses ≥1.6 mg Mo/kg bw/day). The magnitude of the reported decreases in fetal body weights was substantial, but not dose related (i.e., 32% and 30% decreases at the estimated doses of 8.3 and 16.7 mg Mo/kg bw/day). In contrast, in the present study, no effects on resorptions or fetal body weights were observed at dose levels of up to 40 mg Mo/kg bw/day.

The difference in results between the current study and the Fungwe et al. [7] study may relate to differences in route of administration, duration of exposure, and composition of the diet. These investigators administered the test material in the drinking water whereas the current study gave the test material in the diet, which would be unlikely to result in such a large difference in toxicity. The duration of exposure and timing of exposure were different. In the current study, exposure occurred on GD 6–20, and Fungwe et al. [7] exposed female rats continuously from weaning through GD 21. Thus, it is theoretically possible, although unlikely, that the effects observed in Fungwe et al. [7] were due to exposure prior to implantation.

Interestingly, Fungwe et al. [7] used a semi-purified diet with a low molybdenum content of only 0.025 ppm, whereas the present study used a commercial diet containing 1.8 ppm of molybdenum. In addition to the very low molybdenum content of the Fungwe et al. diet, the copper concentration was lower in the semi-purified diet (6.3 ppm) compared to the concentration of copper in the commercial diet (11.6 ppm). A minimum dietary copper concentration of 8 ppm is recommended for pregnant rats [21]. The lower copper concentration in the semi-purified diet in the Fungwe et al. [7] study, together with deficiencies of other constituents or nutrients of a semi-purified diet, may have contributed to the observed toxicity in the Fungwe et al. [7] study.

A major deficiency in the publication by Fungwe et al. [7] is that the authors only state the concentrations of molybdenum added to the drinking water and did not report the dose levels actually consumed in mg Mo/kg bw/day. It is not possible to calculate the intakes accurately since neither the water intakes, nor the body weights of the rats are reported. They quote in their paper that the molybdenum intake by the pregnant rats from water per week in the controls, 5, 10, 50 and 100 mg Mo/L dose groups were “0, 0.82, 1.65, 7.85 and 17.64 mg/L”. The intake units of mg/L are clearly incorrect, so we do not know if these figures should relate to intake per rat, per 100 g, or per kilogram bodyweight. Vyskocil and Viau [4] in a review of the paper, made a variety of assumptions about the probable rat bodyweights and reported intakes and arrived at estimated intake levels of 0, 0.9, 1.6, 8.3, and 16.7 mg Mo/kg bw/day in the controls to top dose levels, respectively. In the absence of better published data the suggested intakes of 0.9 and 1.6 mg Mo/kg bw/day have been used extensively worldwide by regulatory authorities in setting the NOAEL and LOAEL values for molybdenum.

An interesting comparison however can be made between the tissue levels of molybdenum found in the Fungwe et al. [7] study and the current study. Data on molybdenum concentrations in maternal placenta and liver are presented in an earlier publication based on the same study [22]. Table 5 shows the placental and liver concentrations of molybdenum found at termination on GD 21 in the Fungwe study, and the corresponding data from the current study on GD 20. The similarity of the tissue levels in the two studies, suggests that the actual intakes in the Fungwe study may have been about 3 or more times higher than was estimated by Vyskocil and Viau [4].

Previous toxicity studies of molybdate in rodents have reported that high doses of molybdate produced toxicity, which is associated with very high blood and tissue levels of copper. The copper, however, is tightly bound to carrier proteins in the blood and tissues, and it has been suggested that, despite the elevated copper levels, the toxicity of molybdate was actually related to a reduction in bioavailable copper [23–26].

Copper levels in maternal placenta and liver were also measured in both the current study and by Fungwe et al. [22]. Both studies reported that copper levels in these tissues were markedly increased at increasing levels of exposure to molybdenum. However, the levels of copper in the control groups were markedly different between the two studies. The mean copper concentration in the placentae in the controls was 21.3 μg/g dry weight in the current study, and 138 μg/g dry weight in the Fungwe et al. [22] study. Similarly, in the liver, the mean copper concentration in the control group was 12.8 μg/g in the current study, and 101 μg/g dry weight in the Fungwe et al. [22] study. It is surprising that the copper tissue levels were substantially higher in the controls in the Fungwe et al. [22] study compared to the current study, especially since the same strain of rat (Sprague Dawley), but from different sources, were used in both studies and that the copper concentration in the diet was higher in the current study.

In another developmental toxicity study, Sun Su-Ling et al. [9] reported statistically significant changes in the incidence of live fetuses per litter, fetal body measurements and fetal abnormalities in the offspring of pregnant Wistar rats given "molybdenum acid ammonium salts" by gavage on GD 7–16. However, the specific chemical identity, purity and source of the test material were not provided, and as there are a variety of "molybdenum acid ammonium salts" it is not clear what substance was actually tested. Importantly, the validity of the Sun Su-Ling study is questionable because of clear errors in the published paper, as discussed in the Introduction, so this limits the usefulness of the Sun Su-Ling et al. study.

In conclusion, this paper presents the first developmental toxicity study with a molybdenum substance that is compliant with OECD TG 414 and conducted and reported under GLP principles. The NOAEL for both maternal toxicity and developmental toxicity of molybdenum (given as sodium molybdate dihydrate) in the diet under the conditions of this study in rats is 40 mg Mo/kg bw/day, the highest dose level tested.

The statistically significant and dose related alterations observed in maternal copper levels in serum and tissues are well known after exposure to high levels of molybdenum and are considered as physiological adaptations, not a toxic response at these dose levels of molybdenum.

Conflict of interest

FJM and FMS are consultants to IMOA. SC is an employee of IMOA. AKT works in the molybdenum industry.

Transparency document

The Transparency document associated with this article can be found in the online version.

Acknowledgements

The study was funded by the International Molybdenum Association (IMOA). The study was carried out at RTI International, and we are grateful to Ms. Melissa Marr and her technical staff for their diligent work on this study, and likewise to Justin Zyskowski, DCPAH, at Michigan State University, for the analyses of the elements in the diet, serum and tissues.

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