

Assessment of Molybdenum Toxicity in Humans

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In an attempt to define a tolerable daily intake (TDI) for molybdenum based on a toxicological risk analysis approach, a large literature survey was conducted. In man, absorption of molybdenum after oral intake is in the range of 28–77% and urinary excretion is 17–80% of the total dose. A low order of toxicity of molybdenum compounds has been observed in humans. However, with the available data, it is not possible to calculate any dose–response or dose–effect relationships. Because molybdenum toxicity is associated with copper intake or depleted copper stores in the body, humans who have an inadequate intake of dietary copper or some dysfunction in their copper metabolism that makes them copper-deficient could be at greater risk of molybdenum toxicity. In the absence of relevant human studies, animal studies were evaluated for the derivation of the TDI. Effects of Mo on reproduction and foetal development were found to be critical effects observed in rats and mice. A dose–response relationship was observed in a study by Fungwe *et al.*, with a ‘no observed adverse effect’ level (NOAEL) and a ‘lowest observed adverse effect’ level (LOAEL) of 0.9 and 1.6 mg Mo kg⁻¹ day⁻¹, respectively. Applying uncertainty factors of 10 for intraspecies and 10 for interspecies differences to the NOAEL, a TDI of 0.009 mg Mo kg⁻¹ day⁻¹ was calculated. The TDI is given a medium confidence rating. This TDI is more than double the upper limit of adequate intake for adolescents and adults that was derived from the Mo content of the average diet in the USA. Copyright © 1999 John Wiley & Sons, Ltd.

INTRODUCTION

Molybdenum is an essential trace element for both animals and plants. In mammals, molybdenum is a component of certain metalloflavoproteins, including xanthine oxidase, sulphite oxidase and aldehyde oxidase. In plants, it is necessary for fixing of atmospheric nitrogen by bacteria at the start of protein synthesis. Because of these functions, it is ubiquitous in food.^{1–6}

As summarized by Friberg and Lener,⁴ variations of molybdenum concentrations in foodstuffs, especially plants, are greatly dependent on species and soil characteristics. Generally, high concentrations are found in leafy vegetables and legumes, whereas edible roots have a lower content. Animal products are generally low in molybdenum.

The concentration of molybdenum in urban air is minimal (0.01–0.03 µg m⁻³), but it is present in more than one-third of freshwater supplies.⁴ Worldwide, molybdenum in drinking water has been estimated to be in the range 0.11–6.2⁴ or 0–20 µg l⁻¹, levels that would contribute no more than 20% of the intake.⁷

In certain areas where molybdenum ore is mined, considerable contamination may occur, which can cause concentrations in drinking water to increase by an

order of magnitude or more. For example, in some areas in Colorado, mining effluents raise concentrations to as much as 400 µg l⁻¹.⁷ Thus, in such situations a higher-than-average intake in food and the consumption of 2 l day⁻¹ of drinking water derived from mining effluents could result in the ingestion of more than 1000 µg day⁻¹.^{4,7,8}

Molybdenum is an essential element and because no diets have been devised that are so low in molybdenum as to induce a deficiency, the minimum daily requirement is as yet unknown. A range of 150–500 µg day⁻¹ has been estimated as adequate and safe for adults.⁹

In an attempt to define a tolerable daily intake (TDI) for molybdenum based on a toxicological risk analysis approach, a large literature survey was conducted. The results of this work are presented in this article.

METABOLISM

Water-soluble molybdenum compounds are readily absorbed when ingested. The rate of absorption depends on a number of factors, including the chemical form of molybdenum and the animal species. In animals, absorption varies between 75 and 97% (Table 1).

In man, absorption of molybdenum via the digestive tract after oral intake has been calculated to be in the range 28–77%^{10–12} (Table 1). No data exist regarding the chemical form of molybdenum in human diets or its bioavailability and it is not known whether molyb-

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Table 1. Summary of molybdenum absorption studies in laboratory animals and humans

Species	Form	Route	Absorption (%)	Notes	Source
Rat	⁹⁹ Mo (molybdate)	Oral	97	Measured over 6 h	Kosarek and Winston ⁵⁴
Rat	MoS ₂	Oral-diet	0		Fairhall <i>et al.</i> ¹⁵
Guinea pig	MoO ₃	Oral	88	Measured over 16 h	Fairhall <i>et al.</i> ¹⁵
Pig	(NH ₄) ₂ MoO ₄	Oral	75		Miller <i>et al.</i> ⁵⁵
Human	Unknown	Oral-diet	28, 52	Two subjects	Tipton <i>et al.</i> ⁵⁶
Human	Unknown	Oral-diet	77	Children	Alexander <i>et al.</i> ¹²
Human	Unknown	Oral-diet	28–62		Robinson <i>et al.</i> ¹¹

denum in drinking water is absorbed more rapidly than that contained in food. Little information is available on the influence upon molybdenum absorption of other components of diet, particularly proteins and other trace elements.

Once absorbed, molybdenum rapidly appears in the blood and most organs. Blood molybdenum concentrations for humans are normally about 5 µg l⁻¹ and may range up to 400 µg l⁻¹.¹³ The highest molybdenum concentrations are found in the kidneys, liver and bones of most laboratory animals and humans. The adrenals and omentum in humans and the pancreas in mice have a lower concentration of molybdenum relative to liver and kidney but a higher concentration relative to the other organs. There is no apparent bioaccumulation of molybdenum in animal or human tissues. Very little molybdenum seems to cross the placental barrier. When exposure is withdrawn, tissue concentrations quickly return to normal levels.^{14–19}

Excretion, primarily via the urine, is rapid (Table 2). In laboratory animals, 36–90% of molybdenum is excreted in the urine. In humans, the urinary excretion is 17–80% of the total dose. Studies with various exposures either in the workplace or in their water supply indicate an increase in urinary output with increased exposure. Very little (1% or less) of the

molybdenum excretion is via the bile. The biological half-time for molybdenum in humans has not been studied in detail but the biological half-time varies from a few hours to several days in small laboratory animals (Table 3).

Molybdenum metabolism is related to copper and sulphur metabolism. Molybdenum salts are capable of inhibiting the intestinal absorption of iron and copper through mechanisms that are poorly understood but may involve competition for brush-border receptors and/or (in the case of copper) the formation of copper molybdate or thiomolybdate compounds that are poorly absorbed and do not render the copper available for incorporation into ceruloplasmin and other copper-containing proteins. Also, molybdenum may indirectly increase the formation of copper sulphide because molybdenum lowers the activity of sulphide oxidase, leading to the accumulation of larger quantities of copper sulphide and possibly other sulphides as well. Copper generally has a beneficial effect on the symptoms caused by excessive molybdenum, but the action of sulphur compounds, especially sulphate, is not yet clearly understood. Both positive and negative effects have been reported, depending upon the copper status and animal species. Generally, sulphate alleviates molybdenum toxicity in monogastric animals (except when

Table 2. Summary of molybdenum excretion studies

Species	Form	Route	Dose (mg Mo kg ⁻¹)	Excretion (% dose)			Source
				Urine	Faeces	Bile	
Rat	Na ₂ MoO ₄ ^a	Oral	93	84	13		Neilands <i>et al.</i> ³⁷
Rat	(NH ₄) ₂ MoO ₄ ^a	Oral	2–21 000	90	1–2		Bibr and Lener ⁵⁷
Rat	MoO ₃ ^a	Oral	Trace	58	8		Arrington and Davis ⁵⁵
Rat	Na ₂ MoO ₄ ^a	i.v.	4.6			1	Lener and Bibr ⁵⁸
Mouse	(NH ₄) ₂ MoO ₄ ^a	i.v.	Trace	36	3		Rosoff and Spencer ¹⁴
Pig	Na ₂ MoO ₄ ^a	Gavage	12–22	55	8		Shirley <i>et al.</i> ¹⁷
Pig	(NH ₄) ₂ MoO ₄ ^a	i.v., oral	Trace	>75	<15		Bell <i>et al.</i> ⁵⁹
Human	Unknown	Oral-diet	210 and 460 ^c	28 and 52	22 and 42		Tipton and Stewart ⁶⁰
Human	Unknown	Oral-diet	48–96 ^c	29–57	38–72		Robinson <i>et al.</i> ¹¹
Human	Unknown	Oral-diet	43–80 ^c	17–33	56–70		Engel <i>et al.</i> ⁶¹
Human	Unknown	Oral-diet	99–460 ^c	27–79 ^b	21–73 ^b		Schroeder <i>et al.</i> ¹⁹
Human	(NH ₄) ₂ MoO ₄ ^a	i.v.	Trace	24–29	1–7		Rosoff and Spencer ⁶²

^aLabelled molybdenum (⁹⁹Mo).

^bPercentage of total excretion.

^cMean daily intake (µg Mo/per subject).

Table 3. Biological half-times of molybdenum in animals

Species	Form	Route	Dose ($\mu\text{g kg}^{-1}$)	Observation period	Half-time	Compartment	Source
Rat	(NH ₄) ₂ MoO ₄ ^a	s.c. Intra-gastric	<3	2 weeks	47 h	Whole body	Bibr and Lener ⁵⁷
Rat	(NH ₄) ₂ MoO ₄ ^a	s.c.	>3	2 weeks	3 h	Whole body	Bibr and Lener ⁵⁷
Rat	(NH ₄) ₂ MoO ₄ ^a	Intra-gastric	>3	2 weeks	6 h	Whole body	Bibr and Lener ⁵⁷
Rat	(NH ₄) ₂ MoO ₄ ^a	s.c.	800	14 days	3.6 h	Liver	Bibr <i>et al.</i> ¹⁸
Rat	(NH ₄) ₂ MoO ₄ ^a	s.c.	20–1000	14 days	2.5–4.7 days	Kidney, liver, spleen, skin	Bibr and Lener ⁶³
Rat	(NH ₄) ₂ MoO ₄ ^a	s.c.	325 or 670	10 days	7 days	Blood	Kselikova <i>et al.</i> ⁶⁴
Rabbit	No information	No information	No information	No information	0.6 h ^b 16.3 h ^c	Liver	Anonymous ²⁹
Rabbit	No information	No information	No information	No information	0.4 h ^b 12.1 h ^c	Kidney	Anonymous ²⁹
Rabbit	Na ₂ MoO ₄ ^a	i.v.	22 000–25 000 ^d	72 h	19 h	Body	McCarter <i>et al.</i> ⁶⁵

^aLabelled molybdenum (⁹⁹Mo).

^bFirst compartment.

^cSecond compartment.

^dDaily dose for 3 weeks.

they are copper deficient, in which case it can intensify the toxic symptoms) and exacerbates it in ruminants.^{4,20,21}

In ruminants, excessive molybdate intake, especially when coupled with a high sulphur intake and the low copper content of forage crops, produces a copper deficiency with its concomitant symptomatology. This probably results from the formation of biologically unavailable cupric thiomolybdate complexes in the intestine which has been a problem in some areas of the world, notably the UK and New Zealand.²¹ In humans and non-ruminants, copper deficiency from excess molybdate intake is very rare because intake of copper usually exceeds that of molybdate. Indeed, a deficiency of molybdenum from insufficient intake relative to copper is more likely.²²

TOXIC EFFECTS

There is considerable variability in the toxicity of molybdenum, depending on the chemical form and the animal species. Generally, soluble compounds are more toxic than insoluble compounds. In many ways the symptoms resemble those of copper deficiency, and treatment with supplemental copper usually reverses them. However, symptoms may be produced where dietary copper is 'normal' but the molybdenum content is considerably higher than 'normal'.

Ruminants are more sensitive to molybdenum than monogastric animals. Very little is known about specific effects on human health and most information has to be based on experiments with animals.

There is no information on lethal doses in humans. The lethal dose for repeated oral administration is 60–333 mg kg⁻¹ day⁻¹ for soluble molybdenum compounds administered to rats, mouse, guinea pigs and rabbits,^{15,23} but only about 3 mg kg⁻¹ day⁻¹ for steers.²⁴ Histological examinations of animals following acute doses generally show damage to the liver and kidney and sometimes to the adrenals and spleen.

A low order of toxicity of molybdenum compounds has been observed in humans. With the available data it is not possible to calculate any dose–response or dose–effect relationships. Sparingly soluble compounds (e.g. molybdenum disulphide, metal, dioxide) are less toxic than those that are more easily soluble (e.g. trioxide, ammonium molybdate). Increased blood uric acid concentrations and gout-like symptoms have been reported among workers exposed to molybdenum in a copper–molybdenum plant,²⁵ as well as among the general population living in an area with high molybdenum and copper contents in soil and vegetables (daily intake of about 0.14 mg Mo kg⁻¹ day⁻¹).²⁶ One study,²⁷ on the contrary, reported decreased blood uric acid among the general population. Increased urinary excretion of copper was observed in subjects with a dietary molybdenum intake of 0.022 mg kg⁻¹ day⁻¹,²⁸ and increased serum ceruloplasmin was observed in a population with a molybdenum intake of 0.007 mg kg⁻¹ day⁻¹.²⁷ Although increased copper excretion and elevated serum ceruloplasmin^{27,28} are not definitive adverse effects, the potential for mineral imbalance must be taken in consideration.

When inhaled, both metallic molybdenum and sparingly soluble molybdenum trioxide have been reported to damage the lungs. A few cases of pneumoconiosis have been reported.^{29,30}

Possible reasons for the low degree of toxicity are the facts that molybdenum is a necessary trace element in the body, functioning in conjunction with some flavoprotein enzymes (xanthine oxidase, aldehyde oxidase, sulphite oxidase), and it is rapidly eliminated in the urine.^{31–34} Because molybdenum toxicity is associated with copper intake or depleted copper stores in the body, humans who have an inadequate intake of dietary copper or some dysfunction in their copper metabolism that makes them copper deficient, could be at greater risk of molybdenum toxicity.

As can be seen in Table 4, in laboratory animals the most common response at lower subchronic exposures is a growth-depressing action (2–8 mg Mo kg⁻¹ day⁻¹ in rats and rabbits). Developmental changes

Table 4. The LOAEL and/or NOAEL of soluble molybdates administered orally (force-fed, diet, drinking water) from reported studies

Species ^a	Effects	LOAEL (mg Mo kg ⁻¹ day ⁻¹)	NOAEL (mg Mo kg ⁻¹ day ⁻¹)	Duration of exposure	Source
Rat (f)	Prolonged oestrus cycle, decreased gestation weight, effect on embryogenesis	1.6	0.9	9 weeks	Fungwe <i>et al.</i> ⁴⁵
Rat (m)	Growth depression	2		13 weeks	Jeter and Davies ⁴⁷
Rat (m, f)	Bone deformities	7.5		6 weeks	Miller <i>et al.</i> ⁴⁸
Rat (f)	Growth depression	8	2	13 weeks	Jeter and Davies ⁴⁷
Rat (m)	Infertility	8	2	13 weeks	Jeter and Davies ⁴⁷
Rat	Anaemia	50 ^b		5 weeks	Ostrom <i>et al.</i> ³⁶
Rat (m, f)	Diarrhoea	50		5 (m) or 8 (f) weeks	Cox <i>et al.</i> ⁶⁶
Rat (m)	Renal failure	80	40	8 weeks	Bompart <i>et al.</i> ⁴⁹
Rabbit	Reduced growth + histological changes in kidney and liver	5	0.5	6 months	Asmangulyan ³³
Rabbit	Reduced growth, skeletal abnormalities, anaemia	23	46	4 months	Arrington and Davis ²³
Rabbit	Skeletal abnormalities, anaemia	25 ^b		5 weeks	McCarter <i>et al.</i> ³⁹
Rabbit	Thyroidal injury	66		1 month	Widjakusuma <i>et al.</i> ⁶⁷
Guinea pig	Reduced growth	75		8 weeks	Arthur <i>et al.</i> ⁵⁰
Mouse (m, f)	Failure to breed, deaths of offspring and litters	1.5		Three generations	Schroeder and Mitchener ⁵¹

^am = males; f = females.

^bBased on the combination of data from two or more studies examining the reported effect.

were observed at 1.6 mg kg⁻¹ day⁻¹ in rats. At higher doses (8–50 mg Mo kg⁻¹ day⁻¹ in rats; 23 mg Mo kg⁻¹ day⁻¹ in rabbits), symptoms such as male infertility, testicular degeneration, diarrhoea, anorexia and weight loss may occur. An effect that is shared by a number of species involves bone or joint abnormalities (≥ 7.5 mg Mo kg⁻¹ day⁻¹ in rats; 25 mg Mo kg⁻¹ day⁻¹ in rabbits).

Because the weight loss is usually accompanied by anorexia, it has been suggested that the weight loss is due to reduced food intake rather than some other metabolic process. However, pair-feeding experiments demonstrated that reduced food intake was not the primary cause.³⁵

There are some contradictory reports concerning haematological effects in rats. Although one study³⁶ did report anaemia in rats receiving 50 mg Mo kg⁻¹ day⁻¹ in their diet, other studies^{37,38} have failed to reproduce this result in rats receiving 50–500 mg Mo kg⁻¹ day⁻¹. On the other hand, there is a rather consistent picture of haematological effects in rabbits. Several authors^{23,36,39,40} have reported decreased haemoglobin and haematocrit in rabbits receiving ≥ 25 mg Mo kg⁻¹ day⁻¹ in the diet.

Owing to a lack of sufficient evidence on the carcinogenic potential of molybdenum in animals or humans, any conclusions on the potential carcinogenicity and mutagenicity of molybdenum are limited. Molybdenum has not been evaluated for evidence of human carcinogenic potential, either by the US Environmental Protection Agency (USEPA)⁴¹ or by the American Conference of Governmental Industrial Hygienists

(ACGIH)⁴² Molybdenum is not on the list of 'unequivocally proven and justifiably suspected carcinogenic compounds' in Germany.⁴³

KEY HUMAN AND ANIMAL STUDIES

Human studies

Aching joints and symptoms resembling gout have also been reported from areas of Armenia where the population has a high intake of molybdenum via food.²⁶ In the soil, levels of 77 mg Mo kg⁻¹ and 39 mg Cu kg⁻¹ were reported. On the basis of copper and molybdenum levels in different food products, the daily intake in the exposed area was calculated to be 10–15 mg of molybdenum (0.14–0.21 mg kg⁻¹ day⁻¹, assuming the body weight of an adult to be 70 kg) and 5–10 mg of copper, compared with 1–2 mg of molybdenum and 10–15 mg of copper in a control area. Humans and livestock displayed abnormally high serum uric acid levels (the mean level in the symptomatic individuals was 81 mg l⁻¹) and tissue xanthine oxidase activities. Symptomatic individuals also demonstrated hyperuricosuria and elevation in mean blood molybdenum content (310 μ g l⁻¹). Both serum molybdenum and serum xanthine oxidase were positively correlated with serum uric acid levels. Serum uric acid levels increased with increasing residency time in the region; they increased from 37.5 mg l⁻¹ for 1 year up to 64 mg l⁻¹ after 1–5 years and 68 mg l⁻¹ for 5 years

and more. This study, however, has some weaknesses as mentioned by the US National Research Council.⁴⁴ The mean blood copper level was significantly decreased in the sick subjects ($1130 \mu\text{g l}^{-1}$), but the mean copper levels of the controls seemed elevated by current standards at $1830 \mu\text{g l}^{-1}$, suggesting external contamination during the assay procedure. Similar consideration may pertain to the urinary Cu levels ($120 \mu\text{g 24 h}^{-1}$ for the control subjects). Furthermore, the control group consisted of only five subjects, compared with 52 exposed subjects. The US National Research Council⁴⁴ concluded that involvements of molybdenum are speculative and that there was a need to establish a cause-and-effect relationship.

Increased serum concentrations of uric acid and ceruloplasmin (copper-containing protein) were noted in one study of 25 workers (average age: 30 years) in a factory where molybdenum oxide was produced from molybdenum sulphide and where the time-weighted average air concentration was 9.5 mg Mo m^{-3} .²⁵ Workers who had an average employment time of 4 years complained of aching joints and headaches to a greater extent than did a control group. The minimum daily dose of molybdenum as dust has been calculated to be 10.2 mg. Owing to the high worker turnover, epidemiological studies were difficult to conduct properly, making the description of possible harmful effects impossible to assess.

In a well-conducted study by Deosthale and Gopalan,²⁸ the effect of three doses of molybdenum on uric acid and copper excretion was studied in four volunteers. The urinary excretion of uric acid was unaltered at molybdenum intake levels up to $22 \mu\text{g kg}^{-1} \text{ day}^{-1}$.

In another well-conducted study from 1979, Chappell *et al.*²⁷ compared individuals from two cities with low and high levels of molybdenum in the drinking water. The daily molybdenum doses were adequately estimated. The molybdenum intake in the exposed group was $\geq 7 \mu\text{g Mo kg}^{-1} \text{ day}^{-1}$. In contrast to previous studies, lower concentrations of serum uric acid were found in the exposed group in comparison with a control group. However, only two molybdenum doses were compared (exposed and controls).

Animal studies

There are a few studies in which a dose-response relationship was presented and these are reported as appropriate in Table 4. All other published subchronic or chronic studies reporting a given effect on a given animal species were examined and the lowest dose causing this effect in any of these studies was also reported as the 'lowest observed adverse effect' level (LOAEL) in Table 4. None of these studies reported a quality control procedure for the analyses performed.

We recommend the following studies with good scientific design. Because there are not enough chronic studies upon which a good risk assessment can be based, we recommend several subchronic studies with good scientific design. Many of these studies were conducted more than 25 years ago.

In a recent study, Fungwe *et al.*⁴⁵ investigated the effect of supplemental molybdenum on oestrus activity, fertility and foetal development. Weanling female rats

(21 animals per group) were given deionized water containing either no molybdenum or molybdenum at concentrations of 5, 10, 50 and 100 mg l^{-1} as sodium molybdate. Their exposure continued until the 21st day of gestation. The authors reported the corresponding weekly molybdenum intakes of 0.64, 1.12, 5.81 and 11.66 mg per rat , respectively. Assuming an average rat weight of 0.1 kg,⁴⁶ these intakes correspond to molybdenum doses of 0.91, 1.6, 8.3 and $16.7 \text{ mg Mo kg}^{-1} \text{ day}^{-1}$, respectively. Molybdenum supplementation did not appear to affect fertility in the rat and significantly prolonged the oestrus cycle when fed at doses of $\geq 10 \text{ mg l}^{-1}$. Gestational weight gain was higher for the controls and those given 5 mg Mo l^{-1} than for the $10\text{--}100 \text{ mg l}^{-1}$ treatments. Histological data suggested that doses of $\geq 10 \text{ mg l}^{-1}$ delayed foetal oesophageal development, transfer of foetal haemopoiesis to bone marrow and myelination in the spinal cord. Intrauterine deaths were few, but the rate of foetal resorption increased with supplementation at $\geq 10 \text{ mg l}^{-1}$. The results suggest that supplemental Mo may influence oestrus activity and embryogenesis. The 0.9 and $1.6 \text{ mg Mo kg}^{-1} \text{ day}^{-1}$ doses represent a 'no observed adverse effect' level (NOAEL) and a LOAEL, respectively, based on oestrus cycle, gestation weight and foetal development. This study has a very good scientific design and adequate dose-response information.

In the study of Jeter and Davies⁴⁷ from 1954, four molybdenum doses (20, 80, 140 and $700 \text{ mg Mo kg}^{-1}$ in ration) were compared in rats exposed to molybdenum in the diet for up to 13 weeks. The study has an appropriate scientific design. However, no age or body weight of rats at the beginning of experiment were shown (only average weight gain at 11 weeks was reported), so estimation of daily intake is approximate. Assuming an average rat weight of 0.1 kg (from reported data) and 10 g day^{-1} food consumption,⁴⁶ 20, 80, 140 and $700 \text{ mg Mo kg}^{-1}$ in ration corresponds to a molybdenum dose of 2, 8, 14 and $70 \text{ mg kg}^{-1} \text{ day}^{-1}$. The 2 and $8 \text{ mg Mo kg}^{-1} \text{ day}^{-1}$ doses represent a LOAEL for male and female rats, respectively, and the $2 \text{ mg Mo kg}^{-1} \text{ day}^{-1}$ dose represents a NOAEL for female rats based on growth depression and male fertility.

Miller *et al.*⁴⁸ studies the effect of two doses of molybdenum in the diet (75 and $300 \text{ mg Mo kg}^{-1} \text{ day}^{-1}$) on the skeletal system and growth of rats exposed for only 6 weeks. Even for this short period, the study is well done and the results are clear. The experiments are well described, allowing a good estimation of molybdenum daily intake of 7.5 and $30 \text{ mg Mo kg}^{-1} \text{ day}^{-1}$ (the same assumption as in previous study). The study suggests a LOAEL of $7.5 \text{ mg Mo kg}^{-1} \text{ day}^{-1}$ based on body weight loss and bone deformities.

Bompart *et al.*⁴⁹ investigated the effect of molybdenum on renal function. Male rats were given two doses of molybdenum (40 or $80 \text{ mg Mo kg}^{-1} \text{ day}^{-1}$) by gastric intubation for 8 weeks. The study has an appropriate scientific design and the results are adequately evaluated. However, high doses were required to induce a significant effect, and the nephrotoxicity of molybdenum remained moderate when compared to other heavy metals. This study suggests a NOAEL of $40 \text{ mg Mo kg}^{-1} \text{ day}^{-1}$ and a LOAEL of

80 mg Mo kg⁻¹ day⁻¹ based on body weight loss and nephrotoxicity.

Asmangulyan³³ studied the effects of molybdenum in rabbits receiving four different oral doses of molybdenum (0.025, 0.5, 5 or 50 mg Mo kg⁻¹ day⁻¹) for 6 months. The exact daily doses were reported. However, the analytical methods are not well reported (no references, no sufficient description). This experiment suggests that a dose of 0.5 mg Mo kg⁻¹ day⁻¹ may be considered a NOAEL and a dose of 5 mg Mo kg⁻¹ day⁻¹ may be considered a LOAEL in rabbits, based on body weight loss and histological changes in kidney and liver.

In the study of Arrington and Davis,²³ rabbits were fed molybdenum in a commercial ration at five different doses (140, 500, 1000, 2000 and 4000 mg Mo kg⁻¹ in ration) for a period of 4 months. The study contains adequate dose-response information. The scientific design of the study is also adequate. This study suggests a NOAEL and LOAEL of 23 and 46 mg Mo kg⁻¹ day⁻¹ (assuming an average weight of 1.3 kg from reported data and a daily food consumption of 60 g⁴⁶) based on body weight loss, skeletal abnormalities and anaemia.

In the study of Arthur,⁵⁰ different molybdenum doses were used in the diet of guinea pigs treated for 8 weeks. Molybdenum was increased to 8000 mg Mo kg⁻¹ diet in increments of 1000 mg Mo kg⁻¹. The study has an adequate scientific design, both sexes were used and the weight of animals at the start of experiment was shown. Assuming that a guinea pig weighing 0.4 kg consumes 30 g of food daily,⁴⁶ 1 mg kg⁻¹ in the food corresponds to 0.075 mg kg⁻¹ day⁻¹. The level of 75 mg Mo kg⁻¹ day⁻¹ represents a LOAEL in this study, based on loss of copper, growth depression and achromotrichia.

Schroeder and Mitchener⁵¹ studied the effect of one dose of molybdenum (10 mg l⁻¹ as molybdate) in drinking water on the reproduction of mice through three generations. The water consumption was not reported, therefore the daily molybdenum intake can be estimated only approximately. Assuming that a 20 g mouse would consume 3 ml of water per day,⁵² 10 mg Mo l⁻¹ in the water would correspond to 1.5 mg Mo kg⁻¹ day⁻¹. This study indicates a possible reproductive toxicity (some early deaths of offspring, dead litters, maternal deaths, failure to breed) of molybdenum at the dose of 1.5 mg Mo kg⁻¹ day⁻¹.

DERIVATION OF TDI FOR CHRONIC EXPOSURE TO MOLYBDENUM

Principal study and derivation of TDI

In the absence of relevant human studies, animal studies were evaluated for the derivation of TDI. Guinea pigs appeared to be less sensitive than rats, mice and rabbits to molybdenum toxicity. Effects of molybdenum on reproduction and foetal development were found to be critical effects observed in rats and mice. A dose-response relationship was observed in a study by Fungwe *et al.*,⁴⁵ with NOAEL and LOAEL of 0.9 and 1.6 mg Mo kg⁻¹ day⁻¹, respectively.

An uncertainty factor of 10 is used for intraspecies differences. A well-established antagonism between molybdenum and copper may account for a conditioned copper deficiency, causing an inadequate supply of copper to the developing embryo. This factor is used for the protection of sensitive human subpopulations because humans who have an inadequate intake of dietary copper or some dysfunction in their copper metabolism that makes them copper deficient could be at greater risk of molybdenum toxicity.

A factor of 10 was used for interspecies differences because: results of reproductive and/or developmental studies of human exposure are not available, in spite of relevancy of animal results to humans; and data on the toxicokinetics of molybdenum in humans are not complete for comparison with animal toxicokinetic data.

This study has the character of a chronic study because exposure of the developing foetus was in essence chronic (rats were exposed during all the gestation), so no factor for less than chronic exposure was used. An uncertainty factor for an incomplete database is not considered necessary because the database contains supporting studies on more species and with different types of bioassays (see Table 4). Applying the uncertainty factors (10 × 10 = 100) to a NOAEL of 0.9 mg Mo kg⁻¹ day⁻¹, we calculate a TDI of 0.9/100 = 0.009 mg Mo kg⁻¹ day⁻¹.

Supporting studies

A study by Schroeder and Mitchener⁵¹ indicates the possible reproductive toxicity (some early deaths of offspring, dead litters, maternal deaths, failure to breed) of molybdenum in mice at a dose of 1.5 mg Mo kg⁻¹ day⁻¹. Unfortunately, it was the only dose used in this study. In a study by Jeter and Davis,⁴⁷ infertility in male rats was observed after 13 weeks of exposure to 8 mg Mo kg⁻¹ day⁻¹ (LOAEL). The NOAEL in this study was 2 mg Mo kg⁻¹ day⁻¹. In the same study, the authors observed growth depression in female and male rats at 8 and 2 mg Mo kg⁻¹ day⁻¹, respectively. Finally, Asmangulyan³³ observed reduced growth and histopathological changes in kidney and liver of rabbits exposed for 6 months to 5 mg Mo kg⁻¹ day⁻¹ (LOAEL). The NOAEL in this study was 0.5 mg Mo kg⁻¹ day⁻¹.

Confidence in the oral TDI

The level of confidence in the key study is high. This study was well designed with an adequate sample size and provided adequate toxicological endpoints. The level of confidence in the database, lacking data on reproductive and/or developmental effects in humans, and in the oral TDI for molybdenum is medium.

CONCLUSION

In the absence of relevant human studies, the oral TDI of 9 µg Mo kg⁻¹ day⁻¹ for chronic exposure to molybdenum was derived from the NOAEL determined in the study by Fungwe *et al.*⁴⁵ Effects on reproduction

and foetal development were designed as critical effects. The TDI is given a medium confidence rating. This dose is nearly double the corresponding reference dose of $5 \mu\text{g Mo kg}^{-1} \text{ day}^{-1}$ calculated by the EPA.⁴¹ It must be noted that a subchronic human study by Kowalsky *et al.*²⁶ served as the basis for their assessment, in spite of the fact that the latter study has several important weaknesses as described above.

The Food and Nutrition Board of the Subcommittee on the Tenth Edition of *Recommended Dietary Allowances* has established estimated safe and adequate daily intake (ESAADI) values for molybdenum of $2.5\text{--}4.45 \mu\text{g kg}^{-1} \text{ day}^{-1}$ for infants, $1.95\text{--}5.36 \mu\text{g kg}^{-1}$

day^{-1} for children and $1.5\text{--}3.6 \mu\text{g kg}^{-1} \text{ day}^{-1}$ for adolescents and adults⁵³ (cited in EPA⁴¹). Our TDI is more than double the upper limit of adequate intake for adolescents and adults that was derived from the molybdenum content of the average diet in the USA. It can be concluded that our TDI supports the ESAADI recommendations for all groups of the population.

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