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Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Molybdenum

(expressed on 19 October 2000)

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FOREWORD

This opinion is one in the series of opinions of the SCF on the upper levels of vitamins and minerals. The terms of reference given by the European Commission for this task, the related background and the guidelines used by the Committee to develop tolerable upper intake levels for vitamins and minerals used in this opinion, which were expressed by the SCF on 19 October 2000, are available on the Internet at the pages of the SCF, at the address: http://www.europa.eu.int/comm/food/fs/sc/scf/index_en.html.

1. INTRODUCTION

Molybdenum (Mo) is widely distributed in nature, the crustal abundance being 1.5 mg Mo/kg. Molybdenite (MoS_2) is the major source for industrial production of molybdenum compounds. It is used in the manufacture of high strength steel, in electrical equipment, in catalysts and molybdenum pigments. Mo compounds are also used in agriculture for direct seed treatment or in fertiliser formulations (Patty's, 1981; WHO, 1996b).

Mo exists in several valency states, e.g. $Mo^{II}O$, $Mo^{IV}S_2$, $Mo^{VI}O_3$, and as the stable salts $(NH_4)_2Mo^{VI}O_4$ (ammonium molybdate), $(NH_4)_6Mo^{VI}{}_7O_{24}.4H_2O$ (ammonium molybdate tetrahydrate) and $Na_2Mo^{VI}O_4.2H_2O$ (sodium molybdate dihydrate). These latter salts are used in food preparations for special medical purposes (IDACE, 1995).

Mo is ubiquitous in food and water as soluble molybdates. Mo-containing enzymes are found in many plants and animal organisms. In plants and lower organisms these enzymes are involved in the bacterial fixation of N_2, in the conversion of NO_3 to NH_3, in protein synthesis and in some redox reactions. In human and animal tissues the enzymes xanthine dehydrogenase (XD)/oxidase (XO), aldehyde oxidase (AO) and sulfite oxidase (SO) require molybdopterin as cofactor and part of the enzyme molecule. In molybdopterin Mo is bound by two S atoms to the pterin. The redox potential of Mo^V/Mo^{VI} is appropriate for the electron exchange with flavinmononucleotides. Mo is therefore an essential component of flavin- and Fe-containing enzymes (WHO, 1996a).

This evaluation covers those forms of Mo which are found naturally in food and water, as well as soluble molybdates added to foods.

2. NUTRITIONAL BACKGROUND

2.1 Levels in food

Good food sources of Mo are sorghum, leafy vegetables (levels depending on soil content, those grown on neutral or alkaline soil are rich in Mo, those grown on leached acid soil are Mo deficient (WHO, 1996), legumes (beans), grains (cereals, wheat germ), organ meats (liver, kidney), milk and eggs (Rajagopalan, 1987; SCF, 1993). Some 40% of Mo in cereals is lost on milling (Rajagopalan, 1987; SCF, 1993). Fruits, root vegetables, and muscle meat are poor sources (SCF, 1993). High concentrations have been found in shellfish. Soft tissue of fish contain about 1 mg Mo/kg, vascular plants 0.03-5 mg Mo/kg (Patty's, 1981). Mo levels in drinking water range from 0-68 µg/L, but usually do not exceed 10 µg/L (WHO, 1996b).

2.2. Intake estimates

Estimates of daily intake vary widely regionally depending on the soil type. For adults, the representative range of mean estimates of Mo intakes in different countries is 80-250 µg/day and analysis of representative total diets from 11 countries yields an average adult intake of approximately 100 µg/day (WHO, 1996). Intakes for breast fed infants (aged 0-3 m) vary, typically, from 0.1-0.5 µg/kg bw/day, children (from weaning to 3 years) from 5-7 µg/kg bw/day, and adolescents/adults from 1.5-2.5 µg/kg bw/day (WHO, 1996a). In mining areas with contaminated drinking water (levels up to 400 µg/L) intakes from food plus 2 L water can reach about 1000 µg Mo/day.

Estimates of Mo intake in USA range from 44-460 µg/day, in The Netherlands from 48-96 µg/day, in Sweden from 44-260 µg/day, in the UK 50-400 µg/day (mean 128 µg/day), in Germany 60-500 µg/day, and in Finland 120-150 µg/day (SCF, 1993; SCF, 1998). Other data quote for the USA 120-240 µg/day, of which cereals supply 30-40%, legumes 5-20% and to which potable water contributes up to 20 µg/L (Rajagopalan, 1987).

2.3. Nutritional requirements

There are no reliable estimates of human requirements for Mo and no recommended intake has been established by the EC other than that current intakes appear to be adequate and safe (SCF, 1993). The US Food and Nutrition Board (FNB, 1989) set a provisional estimated range of safe and adequate daily dietary intakes for Mo of 75-250 µg for adults and older children, based on average reported intakes. The range for other age groups was derived through extrapolation on the basis of body weight, i.e. 15-30 µg for infants 0-0.5 yr, 20-40 µg for infants 0.5-1 yr, and 25-50, 30-75 and 50-150 µg for children aged 1-3, 4-6 and 7-10 yr, respectively (FNB, 1989). WHO (1996a) has estimated (tentatively) that adult human basal requirement for Mo could be approximately 25 µg/day, corresponding to approximately 0.4 µg/kg bw.

Attention must be paid to the known antagonism between Mo, Cu and sulphate noted particularly in animals.

2.4. Molybdenum deficiency

Mo deficiency in humans is unknown under normal dietary conditions. Intakes of 25-50 µg Mo/day were reported to cause no clinical signs of Mo-deficiency but were associated with biochemical changes suggestive of functional deficiency of XO activity, e.g. a doubling of xanthine excretion, a 20% decrease in uric acid excretion after purine load. Decrease in AO activity was noted because of nicotinamide metabolism abnormalities.

A human syndrome suggestive of Mo deficiency occurs in prolonged total parenteral feeding in association with intolerance of cysteine and methionine, manifested by irritability, tachycardia, tachypnoea, nightblindness, encephalopathies and coma (Abumrad *et al*., 1984; SCF, 1993; SCF, 1998). Biochemical indicators are low tissue SO and XO, raised plasma methionine, reduced plasma uric acid, high excretion of thiosulphate, xanthine and hypoxanthine, low excretion of inorganic sulphate. Treatment requires reduction of protein intake especially S-containing aminoacids. Clinical symptoms were totally eliminated by administration of 300 µg ammonium molybdate/day (equivalent to 147 µg Mo/day). A similar symptom complex is seen in the short-bowel syndrome and after ileal resection for Crohn's disease with faecal loss of 350-530 µg Mo/day, requiring 500 µg Mo parenterally/day for correction (IDACE, 1995; WHO, 1996a; Vyskocil and Viau, 1999).

Human Mo deficiency is seen also in a rare autosomal recessive syndrome in infants, where there is a defective hepatic synthesis of Mo-pterin cofactor. This disease is associated with abnormal faeces, feeding difficulties, neurological and developmental abnormalities, mental retardation, encephalopathy and ectopy of the lens. The urinary levels of sulphite, thiosulphate and S-sulpho-L-cysteine are increased and urinary sulphate levels decreased. Death occurs by age 3. This condition is not ameliorated by dietary Mo supplementation because it is the result of a defective gene (SCF, 1993).

Low intakes of Mo have been claimed to be associated with oesophageal cancer in the Transkei and in Henan (China), where low serum, hair and urine Mo levels had been found (WHO, 1996a). Keshan disease (myocardial defects associated with Se deficiency) may be linked to low cereal and drinking water levels of Mo, as the incidence was reduced by using Mo fertilisers. However, high Mo levels in rice, wheat and soya combined with high tissue and hair levels were found in some Keshan endemic areas.

Goats kept on 24 µg Mo/kg dry matter in their feed developed Mo deficiency symptoms characterised by reduced conception rate, increased abortion rate, increased mortality of dams and offspring (WHO, 1996a).

2.5. Functions of molybdenum-containing enzymes

Xanthine dehydrogenase (XD) converts tissue purines, pyrimidines, pteridins and pyridins by oxidative hydroxylation to uric acid as an irreversible process. Its normal action is that of a dehydrogenase, but when reacting with O_2, during proteolysis, freezing/thawing or in the presence of reactive -SH reagents it changes into Xanthine oxidase (XO), which produces free radicals of oxygen known to be involved in tissue damage following physical injury, reperfusion, injury by toxins or Mo excess. Avian XD is stable, hence birds excrete uric acid. Allopurinol oxidises metabolically to alloxanthine, which inhibits XD.

Reduced XD activity is associated with xanthinuria, low urinary uric acid, high blood xanthine levels, high urinary and blood hypoxanthine levels, renal calculi and depositions in muscles with myopathy. Low Mo intake reduces tissue XD activity, however the intake variations from normal diet are insufficient to exert an effect on XD activity, which can cause overt clinical changes. Similarly, a change in the plasma ratio [xanthine + hypoxanthine]/[uric acid] is too unspecific for diagnosing Mo deficiency. Low XD activity can also be due to low protein intake or hepatoma, while high XD activity can be due to high protein intake, low vitamin E status, administration of interferon or administration of agents stimulating interferon release. It is not known whether high Mo intake stimulates tissue XD activity (Rajagopalan, 1987; WHO, 1996a).

Aldehyde oxidase is structurally and chemically similar to XO, has a similar tissue distribution and shares some substrates, e.g.: aldehydes, substituted pyridines, pyrimidines, quinolines and purine derivatives. Its principal metabolic role is unknown (Rajagopalan, 1987).

Sulphite oxidase (SO) is a haem-containing molybdoprotein located in the intermembraneous space of mitochondria. SO converts sulphite to sulphate. Sulphite derives metabolically from S-amino acids, e.g. cysteine, methionine. SO occurs in the liver of man and other species (WHO, 1996a).

2.6. Kinetics and metabolism in laboratory animals

The rate of gastrointestinal absorption of Mo depends on its chemical nature and the animal species. Ingested Mo^{VI} but not Mo^{IV} is readily absorbed from the duodenum and proximal jejunum. Water-soluble molybdates, thiomolybdates and oxothiomolybdates and Mo in herbage and green vegetables are absorbed to 75-97% by laboratory animals and ruminants. Insoluble MoS_2 is not absorbed, Mo^{IV} compounds are not readily absorbed. Intestinal absorption is inhibited by high intraluminal sulphate concentrations, probably because of competition for the common carrier. Silicates also inhibit the absorption of dietary molybdates.

Absorbed Mo rapidly appears in the blood loosely attached to the erythrocytes, specifically bound to α2-macroglobulins (IDACE, 1995). In rodents it is distributed mainly to the liver, converted to molybdate and 36-90% of the total dose is excreted in the urine, less than 1% in the bile and only some in the faeces (IDACE, 1995). In rabbits and guinea pigs Mo is deposited in the tissues within 4 hours after initial high blood and bile levels and eliminated within 72 hours by the kidneys. In horses, cattle and sheep faecal elimination is about half the urinary elimination because of limited absorption. Some bone storage was noted (Patty's, 1981). Mo crosses the placenta. Sulphate reduces the utilisation of Mo by some tissues and increases the urinary Mo excretion (Patty's, 1981; WHO, 1996a). Mo is reabsorbed by the renal tubules but this reabsorption is reduced by S-containing and by acid proteins. The reabsorbed Mo deposits in liver, lung, bone and skin. It is responsible for F storage and aids retention of F in the bone of old rats as well as decreasing caries in rats (Patty's, 1981; Casarett, 1975). Small amounts of Mo increase antibody formation, e.g. agglutinins (Patty's, 1981).

^{99}Mo injected into dogs was concentrated in liver, kidney, pancreas, pituitary, thyroid and adrenals but none appeared in brain, white marrow or fat (Patty's, 1981). The biological half-life varies from a few hours to several days in small laboratory animals and is related to the Cu and S metabolism.

2.7. Kinetics and metabolism in humans

Water-soluble Mo compounds and Mo in herbage and green vegetables are absorbed by man from 40-50% (WHO, 1996a). The absorption rate from drinking water may be the same as from food. Twenty five percent of absorbed Mo appears rapidly in the blood loosely attached to the erythrocytes, specifically bound to α2-macroglobulins (IDACE, 1995), normal blood levels being 2-6 µg/L whole blood or 0.55 µg/L serum. In man, the highest levels appear in kidney, liver and bone, raised levels appear also in adrenals, fat and omentum. There is no bioaccumulation, tissue levels rapidly returning to normal once exposure stops. Increased exposure at the work place or through drinking water is balanced by increased urinary excretion.

16-27% of i.v. administered ^{99}Mo to man was excreted in 5 days in the urine. Faecal excretion over 10 days was 1-7%. Mo was rapidly cleared from the blood within 24 hours (Patty's, 1981).

Data on the Mo status of normal tissues are unreliable. Quoted blood and serum levels vary by 4 orders of magnitude. Serum levels of Mo rise in liver functional defects, hepatitis, hepatic tumours and after certain drugs. Raised blood levels are seen in uraemia, rheumatic disorders and CVS disease. Human liver contains 1.3-2.9 mg Mo/kg dry matter, kidney 1.6 mg/kg dry matter, lung 0.15 mg/kg dry matter, brain and muscle 0.14 mg/kg dry matter, hair 0.07-0.16 mg/kg (WHO, 1996a).

2.8. Relationship between molybdenum, copper and sulphate

The relationship between Mo, Cu and sulphate is complex and varies with the species considered (Mills and Fell, 1960; Arthur, 1965; Huber *et al.*, 1971; Casarett and Doull, 1975; Nishioka, 1975; Patty's, 1981).

3. HAZARD IDENTIFICATION

The insoluble compounds MoS_2, MoO_2, and Mo metal are less toxic than the more soluble molybdates. The oral LD_{50} for molybdates in rats lies between 101-330 mg Mo/kg bw (Patty's, 1981). The lethal repeated oral dose for mouse, guinea pig and rabbit lies between 60-330 mg Mo/kg bw (Mills and Davis, 1987). No syndrome of industrial toxicity due to handling of molybdenum compounds is known in humans, but the chronic inhalation of 4 mg Mo/m^3 as MoO_3 for 4 years was associated with pneumoconiosis (Casarett and Doull, 1975; Vyskocil and Viau, 1999). Signs of human Mo toxicity are diarrhoea, anaemia, immaturity of erythrocytes, uricaemia. Thiomolybdate at levels of 5 mg Mo/kg bw causes in experimental animals diarrhoea, anaemia and skeletal lesions (Mills and Davis, 1987).

Na_2MoO_4 was a primary skin irritant for 24 hrs after application, but the skin lesions had cleared within 72 hrs. A 20% solution caused conjunctival redness but no corneal irritation. There was no sensitisation (Patty's, 1981). Mo released from surgical metal implants can induce a delayed type of hypersensitivity with PUO and ANA-ve systemic lupus erythematosus (positive lymphocyte transformation test) (Federmann *et al.*, 1994).

Chronic small doses of molybdate have been reported to inactivate the glutaminases of brain and liver causing a decrease in ammonia release (Patty's, 1981). Small amounts of molybdate have similarly been reported to impair the intestinal utilisation of carotenes and to reduce vitamin A status (Patty's, 1981).

The evidence for anticariogenicity in man is contradictory and inconclusive. Mo accumulates in teeth and dental enamel. In a study on the cytopathogenicity of Mo against distinct cell types Mo^{5+} was tested *in vitro* against L-929 murine fibroblasts and primary human gingival fibroblasts because of release from dental alloys. Mo^{5+} had only a low potency and a low NOEL, as measured by its effect on DNA pattern and cell ultrastructure, which latter showed necrosis but not apoptosis. It had no effect on human mast cells. The dose range tested was 0.0033-1.0 mmol/L (Schedle *et al.*, 1995).

Rodent cardiomyocyte cultures require Mo and Se for survival, growth and normal electrophysiological function (WHO, 1996a).

3.1. Molybdenosis

In animals, molybdenosis can occur in cattle, sheep and horses by pollution of pasture with fly ash, indicating ready bioavailability of Mo (Ladefoged and Sturup, 1995). Intoxication (known as teart in cattle and sheep) occurs also on feeding forage growing on shales, mineralised granites and some peats, containing 20-100 mg/kg Mo. The symptoms are loss of appetite, listlessness, diarrhoea, poor growth, anaemia with low Hb and RBC and in ruminants are probably due to secondary Cu deficiency. Cattle, rabbit and chicks also develop fatty degeneration of liver and kidneys. The signs are osteogenic defects with skeletal and joint deformities, spontaneous sub-epithelial fractures, mandibular exostoses, reduced AP activity and reduced proteoglycan content of cartilage. In ruminants it is always associated with "conditioned" Cu-deficiency. Typically, anaemia, cardiac hypertrophy, and achromotrichia from defective melanin synthesis are found. In other species, inhibition of phosphoadenosine-phosphosulphate synthesis, oestrus disturbance, testicular degeneration were noted. Mo^{vi} is more toxic to rats and guinea pigs. $(NH_4)_2MoO_4$ in hepatotoxic doses reduced succinic dehydrogenase and cytochrome-c oxidase in rats. Other changes reported were depletion of tissue nicotinamide nucleotides, hyperaminoaciduria, reduction in erythrocyte life span and hypothyroidism, accompanied by low plasma thyroxine and inhibition of thyroid hormone secretion (Patty's, 1981).

In humans high Mo intakes occur with industrial exposure or through food. It is associated with raised XD activity, uricaemia, uricosuria and a higher incidence of gout (IDACE, 1995). In areas with high geological Mo levels the human XO level is increased (IDACE, 1995). Biochemical changes noted were hypoalbuminaemia, a rise in α-globulins, and raised serum bilirubin as sign of hepatotoxicity. It may be associated with oesophageal cancer.

3.2. Toxic effects in animals

Groups of 4 Holtzman rats were fed daily for 6 weeks diets containing 75 mg and 300 mg Mo/kg feed (equivalent to doses of 3.75 mg Mo/kg bw or 15 mg Mo/kg bw). Growth was significantly inhibited and Cu and Mo concentrations in the liver increased, but the addition of sulphate reduced these effects. The femorotibial joints were enlarged and the epiphyses of

femur and tibia were thickened. The LOAEL was 3.75 mg Mo/kg bw for both bodyweight loss and bone deformities (Miller *et al*., 1956; Vyskocil and Viau, 1999).

In an 8 week study in male rats doses of 40 or 80 mg Mo/kg bw/day were given by gavage and the nephrotoxicity investigated. The NOAEL was 40 mg Mo/kg bw/day based on bodyweight loss and nephrotoxicity. The nephrotoxicity was moderate (Bompart *et al.*, 1990; Vyskocil and Viau, 1999).

Weanling Long-Evans rats received in their diet 50 or 80 mg Mo/kg bw over 5-8 weeks. Diarrhoea and reduced weight gain were noted. Hepatic Cu levels increased (Suttle, 1980).

Rabbits were exposed for 6 months to oral doses of 0.025, 0.5, 5, 50 mg Mo/kg bw/day. Body weight loss and histological changes in liver and kidney were noted at doses of 5 mg/kg bw/day and above, the NOAEL being 0.5 mg/kg bw/day. The weakness of the study was the uncertainty of the analytical method (Asmangulyan, 1965; Vyskocil and Viau, 1999).

Rabbits were exposed for 4 months to oral doses of 40, 500, 1000, 2000, 4000 mg Mo/kg feed (equivalent to 1.8, 23, 46, 92, 184 mg Mo/kg bw/day for a 1.3 kg rabbit consuming 60 g feed/day). The NOAEL was 23 mg/kg bw/day based on bodyweight loss, skeletal abnormalities and anaemia (Arrington and Davis, 1953; Vyskocil and Viau, 1999).

Male and female guinea pigs were treated for 8 weeks with doses of molybdenum in their diet rising by increments of 1000 mg to 8000 mg Mo/kg feed (1000 mg/kg feed corresponds to 75 mg Mo/kg bw/day for a guinea pig weighing 400 g and consuming 30 g feed/day). The LOAEL was 75 mg Mo/kg bw/day based on loss of Cu, growth depression and achromotrichia. Guinea pigs appear to be a less sensitive species to large doses of molybdenum (Arthur, 1965; Vyskocil and Viau, 1999).

3.2.1. Carcinogenicity

There are no relevant studies in animals or man. Molybdates are not on the MAK list, EPA list or ACGIH list (Vyskocil and Viau, 1999). Intraperitoneal administration to strain A mice of MoO_3 significantly increased the incidence of lung adenomas (Stoner *et al.*, 1976). Mo has been found to prevent oesophageal, forestomach and mammary cancer induced by N-nitroso compounds in laboratory animals (Luo *et al.*, 1983; Wei *et al.*, 1985).

3.2.2. Genotoxicity

$(NH_4)_6Mo_7O_{24}$ was mutagenic in two of three *Escherichia coli* strains. $MoCl_5$ was negative and $(NH_4)_6Mo_7O_{24}$ positive in the *Bacillus subtilis rec*-assay using strains H17 (repair-competent) and strain M45 (repair-deficient) (Nishioka, 1975). Ammonium and sodium molybdate were neither mutagenic nor recombinogenic in *Saccharomyces cereviseae* reverse mutation and gene conversion assays (Singh, 1983).

3.2.3. Cytotoxicity

In a study on the cytopathogenicity of Mo against distinct cell types (Schedle *et al.*, 1995), Mo^{5+} (dose range from 0.0033 to 1.0 mmol/L) was tested *in vitro* against L-929 murine fibroblasts and primary human gingival fibroblasts because of release from dental alloys. Mo^{5+}

had only a low potency for inhibition of ^{3}H-thymidine incorporation relative to other metal cations. The cytopathogenic effect on both the DNA pattern and the ultrastructure of the cells revealed signs of necrosis but no signs of apoptosis. Mo^{5+} had no effect on human mast cells.

3.2.4. Reproduction and teratogenicity

Four pregnant Cheviot ewes were given in their feed an extra 50 mg Mo/day as ammonium molybdate. Three of the four newborn lambs showed ataxia with histological evidence of cortical degeneration, demyelination of the cortex and spinal cord (Mills and Fell, 1960).

Two male Holstein calves received daily orally by capsules either 4.1 or 7.8 mg Mo/kg bw. Gradual disappearance of spermatogenic and interstitial testicular tissue was noted. The LOAEL was 4.1 mg Mo/kg bw (Thomas and Moss, 1951).

Five pairs of mice (Charles River CD) were given a daily single dose of 10 mg Mo/L (1.5 mg Mo/kg bw) as molybdate in their drinking water for 6 months or about 3 generations. As water consumption was not measured, the calculated daily intake is based on a 20 g mouse consuming 3 ml water/day. Excess pup deaths (15/238) in the F_1 generation and 7/242 pup deaths plus 5 dead litters and 1 maternal deaths in the F_2 generation and infertility were noted. This would correspond to a LOAEL of 1.5 mg Mo/kg bw/day (Schroeder and Mitchener, 1971; Vyskocil and Viau, 1999).

In a 13 week study Long-Evans rats were given in the diet doses of 20, 80, 140, 700 mg Mo/kg feed (calculated to represent approximately 2, 8, 14, 70 mg Mo/kg bw/day for a 100 g rat consuming 10 g feed/day) and either 5 or 20 mg Cu/kg bw additionally. Growth depression was observed at the lowest dose in males, and male fertility was depressed at 14 mg/kg bw/day as shown by fewer litters and degeneration of seminiferous tubules. There was less milk production by females on high dose Mo as pups gained less weight. The LOAEL for growth depression for males was therefore 2 mg/kg bw/day and the NOAEL for infertility of males was 2 mg/kg bw/day. For females the NOAEL for growth depression was 2 mg/kg bw/day (Jeter and Davis, 1954; Vyskocil and Viau, 1999).

In a 9 weeks study in SD rats on the effects of Mo supplementation on oestrus activity, fertility and foetal development, 5 groups, each of 21 female weaning rats, were given for 6 weeks a basic diet containing 0.025 mg Mo/kg diet as well as 6.3 mg Cu/kg diet, and additionally in their drinking water doses of 0, 5, 10, 50 and 100 mg Mo/L as sodium molybdate ($Na_2MoO_4.2H_2O$) for 3 weeks until the 21st day of gestation. Six animals in each group were sacrificed after 6 weeks to determine the oestrus cycle length. The remaining 15 animals in each group were mated with untreated males and allowed to continue gestation for 21 days. The average mean weekly supplementary Mo intakes were 0.0, 0.64, 1.12, 5.81 and 11.56 mg Mo/rat (equivalent to 0, 0.91, 1.6, 8.3 and 16.7 mg Mo/kg bw/day assuming an average rat weight of 100 g). There was no effect on fertility, food and water consumption. Oestrus cycle was prolonged from 1.6 mg/kg bw/day and higher supplementation. Gestational weight, litter size and foetal weights were less than controls for the groups fed 1.6 mg/kg bw/day and higher doses. Histopathology showed delayed histological development of foetal structures, delayed oesophageal development, delayed transfer of foetal haematopoeisis from liver to bone marrow, and delayed myelination of the spinal cord at doses of ≥1.6 mg/kg bw/day. Foetal resorption increased at doses of 1.6 mg/kg bw/day and higher. SO and XDH/XO activity increased with Mo supplementation but less in pregnant animals at dose levels of 1.6 mg/kg

bw/day and above. The NOAEL was 0.9 mg Mo/kg bw/day. The study was well designed. (Fungwe *et al.*, 1990; Vyskocil and Viau, 1999).

3.3. Toxic effects in humans

There are no well-designed chronic studies in man which can be used for risk assessment.

In an area in Armenia, where the population is exposed to a high dietary intake of Mo for geophysical reasons from soil levels of 77 mg Mo/kg and 39 mg Cu/kg, aching joints and gout-like symptoms have been reported. The daily intakes of Mo and Cu, calculated from analysis of levels in different foods, were 10-15 mg Mo/day (equivalent to 0.14-0.21 mg Mo/kg bw/day for a 70 kg adult) and 5-10 mg Cu/day, compared to intakes of 1-2 mg Mo and 10-15 mg Cu in a control area. Biochemical investigations showed abnormally high serum uric acid levels in humans and livestock (81 mg/L in humans with symptoms). Tissue XO activity was also high. Individuals with symptoms had hyperuricosuria and a raised Mo blood level (310 µg/L). Serum molybdate and XO levels were positively correlated with serum uric acid levels. Serum uric acid levels increased with residence time from 37.5 mg/L after 1 year to 68 mg/L after 5 years. Weaknesses of this study were the low blood Cu level of 1130 µg/L in affected persons *vs.* 1830 µg Cu/L in controls (possibly contaminated samples) and the ratio of 5 controls to 52 exposed cases. The US NRC concluded that the involvement of Mo was speculative (Kovalskiy *et al.*, 1961; Vyskocil and Viau, 1999).

In another study on 25 workers exposed for an average of 30 years in a MoS_2 roasting factory to time-weighted average air concentrations of 9.5 mg Mo/m^3 the serum uric acid and ceruloplasmin levels were raised. After 4 years exposure there was a greater incidence of aching joints and headaches than in controls. The minimum daily dose of Mo as dust was calculated as 10.2 mg. The weakness of the study was the high turnover of workers, which made epidemiological assessment impossible (Walravens *et al.*, 1979; Vyskocil and Viau, 1999).

In another study on 4 volunteers three different oral doses of Mo were administered. Urinary uric acid excretion was found to be unchanged up to doses of 22 µg/kg bw/day (Deosthale and Gopalan, 1974; Vyskocil and Viau, 1999).

Serum uric acid levels were compared in individuals of 2 cities with high and low Mo levels in their drinking water. The adequately determined Mo intake of the exposed individuals was ≥7 µg/kg bw/day, yet serum uric acid levels were lower than in the controls. Only two Mo levels were compared (Chappel *et al.*, 1979; Vyskocil and Viau, 1999).

In a study on 4 young men fed dietary doses of Mo varying from 22-1490 µg/day for 24 days ^{100}Mo was fed 5 times, ^{97}Mo was infused 3 times and ^{94}Mo was used for assessing the total Mo content of urine and faeces by isotope dilution. Absorption was found to be 88-93% efficient, especially the larger the dose. Urinary excretion was proportional to the dietary load and slow at low doses. Mo retention appeared to be regulated by urinary excretion. No adverse effects were noted with doses up to 1500 µg/day for 24 days (Turnlund *et al.*, 1995).

In 60 patients on long-term haemodialysis the relationship of serum Mo levels to serum ß2-microglobulin and C-parathyroid hormone levels and to the incidence of arthritis was investigated. Haemodialysis reduced the Mo serum level from 2.7 to 1.4 µg/dl (normal 0.02-

0.13 µg Mo/dl). Serum Mo levels correlated with ß2-MG and C-PTH levels and serum Ca^{2+}. In 9 patients with arthritis the serum Mo levels averaged 12.8 µg/dl, suggesting that Mo accumulation contributes to arthritis (Hosokawa and Yoshida, 1994).

4. DOSE RESPONSE ASSESSMENT

There are no adequate human data for establishing a UL. Growth depression occurs in rats at 2-8 mg Mo/kg bw/day (Jeter and Davis, 1954; Miller *et al.*, 1956) and skeletal changes at 7.5 mg Mo/kg bw/day (Miller *et al.*, 1956). Reproductive and developmental changes were found in rats at 1.6-2 mg Mo/kg bw/day (Jeter and Davis, 1954; Fungwe *et al.*, 1990). In mice infertility and early pup deaths were noted at 1.5 mg Mo/kg bw/day (Schroeder and Mitchener, 1971). In rabbits skeletal changes and nephrotoxicity were found at 5 mg Mo/kg bw/day (Asmangulyan, 1965), while skeletal changes, bodyweight loss and anaemia were seen at 25-46 mg Mo/kg bw/day (Arrington and Davis, 1953; McCarter *et al.*, 1962). Reduced growth occurred in guinea pigs at 75 mg Mo/kg bw/day (Arthur, 1965). Adverse spermatogenic effects were seen in calves at 4 mg Mo/kg bw/day (Suttle and Field, 1969). Thiomolybdate intoxication can occur in experimental animals at intakes of 5 mg Mo/kg bw (Mills and Davis, 1987).

From these studies the critical effects of molybdenum in the rat and mouse appear to be effects on reproduction, particularly foetal development. The pivotal animal study is the 9 weeks study in the rat showing a NOAEL of 0.9 mg Mo/kg bw/day (Fungwe *et al.*, 1990).

5. DERIVATION OF A TOLERABLE UPPER INTAKE LEVEL (UL)

A tolerable upper intake level (UL) can be established using the 9-week study in the rat (Fungwe *et al.*, 1990). This study in rats is pivotal because of its satisfactory design, the use of an adequate number of test animals, demonstration of a clear dose-response relationship and clear toxicological endpoints. The NOAEL of this study was 0.9 mg/kg bw/day for reproductive toxicity. An uncertainty factor of 100 is used. This comprises a factor of 10 for protecting sensitive human sub-populations with inadequate Cu intake or with deficient Cu metabolism in view of the species differences in antagonism between Mo and Cu, and another factor of 10 to cover the lack of knowledge about reproductive effects of Mo in humans and incomplete data on the toxicokinetics in man. Because the exposure in this 9-week rat study is sufficient to cover the relevant period of foetal development, a further uncertainty factor is unnecessary. This provides a UL of approximately 0.01 mg/kg bw/day, equivalent to 0.6 mg/person/day for adults, which also covers pregnant and lactating women.

A further consideration is required in relation to ULs for children, since an adverse effect on growth of young animals was seen in another study in rats (Jeter and Davis, 1954; Vyskocil and Viau, 1999), with a LOAEL of 2 mg/kg bw/day. This indicates that the UL for children should be derived by extrapolating from the adult UL on a body weight basis using the reference body weights for Europe published by the Scientific Committee for Food (SCF, 1993).

Age (years)	UL (mg/day)
1-3	0.1
4-6	0.2
7-10	0.25
11-14	0.4
15-17	0.5

6. CHARACTERISATION OF RISK

The UL is six times the mean estimated intake of 100 µg Mo/day for adults in 11 different countries (WHO, 1996a) and exceeds the upper range of intakes for The Netherlands (96 µg/day), Sweden (260 µg/day), the UK (400 µg/day), Germany (500 µg/day), and Finland (150 µg/day) (SCF, 1993; SCF, 1998). However, in mining areas with contaminated water supplies, drinking water levels may reach up to 400 µg Mo/L. In these circumstances, the daily potential intakes from food and 2 L drinking water could reach 1000 µg Mo/person/day.

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