European Food Safety Authority

*The EFSA Journal* (2009) 1136, 1-21

# SCIENTIFIC OPINION

## Potassium molybdate as a source of molybdenum added for nutritional purposes to food supplements[1]

## Scientific Opinion of the Panel on Food Additives and Nutrient Sources added to Food (ANS)

**(Question No EFSA-Q-2005-157)**

**Adopted on 4 June 2009**

**PANEL MEMBERS**

F. Aguilar, U.R. Charrondiere, B. Dusemund, P. Galtier, J. Gilbert, D.M. Gott, S. Grilli, R. Guertler, G.E.N. Kass, J. Koenig, C. Lambré, J-C. Larsen, J-C. Leblanc, A. Mortensen, D. Parent-Massin, I. Pratt, I.M.C.M. Rietjens, I. Stankovic, P. Tobback, T. Verguieva, R. Woutersen.

**SUMMARY**

Following a request from the European Commission to the European Food Safety Authority (EFSA), the Scientific Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to deliver a scientific opinion on the safety of potassium molybdate, as a source of molybdenum, added for nutritional purposes in food supplements and on the bioavailability of molybdenum from this source.

The present opinion deals only with the safety of potassium molybdate, as a source of molybdenum, added for nutritional purposes in food supplements and with the bioavailability of molybdenum from this source. The safety of molybdenum itself, in terms of the amounts that may be consumed, is outside the remit of this Panel.

Sodium molybdate and ammonium molybdate are already listed in annexes of the Commission Directive 2002/46/EC, as substances which may be used in the manufacture of food supplements, and the Commission Directive 2001/15/EC, as substances that may be added for specific nutritional purposes in foods for particular nutritional uses.

According to the petitioner, potassium molybdate is intended to be used in supplements to provide an intake of 5-20 µg molybdenum/day (2 tablets), which is equivalent to 12-50 µg of potassium molybdate/day.

[1] For citation purposes: Scientific Opinion of the Panel on Food Additives and Nutrient Sources added to Food on potassium molybdate as a source of molybdenum added for nutritional purposes to food supplements following a request from the European Commission. *The EFSA Journal* (2009) 1136, 1-21

Based on an average dietary molybdenum intake in the range of 80-250 µg/day, the Panel estimated that consumption of an additional food supplement containing 20 µg molybdenum/day (the highest use level proposed by the petitioner) would result in a total anticipated dietary exposure between 100 and 270 µg molybdenum/day in an adult at the average level of dietary intake.

When the high dietary intake range of 96-500 µg/day molybdenum for the European population is considered, the consumption of a food supplement providing 20 µg/day would result in a total anticipated exposure of between 116 and 520 µg/day. The Panel notes that this molybdenum exposure would not exceed the Tolerable Upper Intake Level (UL) of 0.6 mg/day as established by the Scientific Committee for Food (SCF) in 2000 for adults.

The Panel considers that the use levels proposed by the petitioner (between 5 and 20 µg/day) would not exceed the ULs of 0.1 to 0.5 mg molybdenum/day defined by the SCF for children (1-17 years old). However, given the wide range of UL values established by the SCF based on age body weights, the Panel considers that in some cases these ULs could be exceeded when molybdenum intake from food is taken into account together with food supplementation.

Assuming a dietary intake range between 64-89 and 80-144 µg molybdenum/day for children between 1-3 and 4-17 years old, respectively, in addition to a daily consumption of an additional food supplement containing 20 µg molybdenum (the highest proposed use level by the petitioner), the Panel estimates total anticipated exposures between 84-109 and 100-164 µg molybdenum/day respectively. The Panel notes that the molybdenum exposure exceeds the UL of 0.1 mg/person/day as established by the SCF in 2000 for children between 1 and 3 years of age.

Assuming a high percentile dietary molybdenum intake of 199 µg/day for age group of 4-6 years old, the Panel estimates that a consumption of an additional food supplement containing 20 µg molybdenum (the highest proposed use level by the petitioner) would result in a total anticipated daily molybdenum intake of 219 µg molybdenum/day at the high level of dietary exposure. The Panel notes that this molybdenum exposure exceeds the ULs of 0.1 mg/person/day and 0.2 mg/person/day, as established by the SCF in 2000 for children between 1 and 6 years old, respectively.

Based on the highest proposed use level, an anticipated exposure to potassium from the food supplements is 16 µg/day. The Panel considered this amount as being negligible compared to the average daily dietary intake of potassium defined by EFSA in 2005, which ranges from 2.7-4 g/day.

No data on bioavailability were provided on the source, potassium molybdate itself. The Panel considers that due to its ionisation properties (pKa = 4), the non-ionized form of potassium molybdate should be absorbed by a diffusion process in the stomach. Potassium molybdate should also dissociate into its constituents, potassium and molybdate ions, in the small intestine. Accordingly, molybdenum from potassium molybdate should be bioavailable and absorbed in the same manner as from other soluble molybdates. Regarding other water-soluble sodium or ammonium molybdates, these compounds are readily taken up through the gastrointestinal tract. Following absorption, molybdates are distributed throughout the body with the highest levels generally found in the liver, kidneys, spleen, and bone. Biological half-life may vary from several hours in laboratory animals to as much as several weeks in humans.

No toxicity data were provided on the source, potassium molybdate, itself.

The toxicity of molybdates has been reviewed by several authorities including the SCF, and the UK Expert Group on Vitamins and Minerals (EVM). Overall these reviews indicate that the data documenting molybdenum toxicity in humans are limited. In studies conducted in a region of Armenia where levels of molybdenum in the soil are high, adults were found to have elevated plasma and urine concentrations of uric acid and gout-like symptoms. In these studies, the daily molybdenum intake for adults was estimated to be 10-15 mg.

In animals, acutely toxic oral doses of molybdenum result in severe gastrointestinal irritation with diarrhea, coma and death from cardiac failure. Subchronic and chronic oral exposures can result in growth retardation, anaemia, hypothyroidism, bone and joint deformities, sterility, liver and kidney abnormalities, and death. In rabbit, two separate subchronic toxicological studies (4 and 6 months respectively) of molybdates indicated differences in the No-Observable-Adverse-Effect-Level (NOAEL) for ammonium and sodium molybdates (0.5 and 23 mg/kg bw/day, respectively). Regarding reproductive toxicity, male sterility and embryotoxic effects of sodium molybdate were observed in rats, and in particularly reduced foetal weight gain, delayed histological development of foetal structures and increased fetal resorption. The NOAEL of this study was 0.9 mg molybdenum/kg bw/day for reproductive toxicity. This study in rats was considered pivotal by the SCF, in its opinion on the tolerable intake level of molybdenum.

There are no relevant studies of molybdenum or molybdate carcinogenicity in animals or humans. Regarding genotoxicity, both negative and positive responses on bacteria have been obtained with molybdates, as previously indicated by the SCF. Ammonium molybdate induced chromosome aberrations and sister-chromatid exchanges in human lymphocytes *in vitro*. Molybdenum trioxide but not ammonium molybdate induced chromosome damage in mouse bone marrow whereas dominant lethal mutations were induced in Drosophila exposed to ammonium molybdate. A study on the tolerable intake level of molybdenum, not available at the time of the SCF opinion was published, used the micronucleus assay in human lymphocytes. In this study, ammonium molybdate was more potent than sodium molybdate in inducing chromosome loss in cultured human lymphocytes. This study also assessed the genotoxicity of sodium molybdate *in vivo* by using the micronucleus assay in mouse bone marrow and the dominant lethal assay in mice, this study was used to assess the genotoxic effects of sodium molybdate *in vivo*. However, it provided a limited evidence of *in vivo* genotoxicity of this salt in mouse somatic and germ cells.

The Panel concludes that the use of potassium molybdate, as a source of molybdenum, added for nutritional purposes in food supplements at the proposed use levels, is of no safety concern provided the applicable UL for molybdenum established by the SCF is not exceeded .

The Panel notes that since the SCF adopted its opinion on molybdenum, new toxicological data have been made available on *in vitro* and *in vivo* genotoxicity of molybdenum that might need further investigation.

**Key words:**

Potassium molybdate, dipotassium tetraoxomolybdate, CAS Registry Number 13446-49-6, food supplement.

## TABLE OF CONTENTS

## BACKGROUND AS PROVIDED BY THE COMMISSION

The European Community legislation lists nutritional substances that may be used for nutritional purposes in certain categories of foods as sources of certain nutrients.

The Commission has received a request for the evaluation of potassium molybdate added for nutritional purposes to food supplements. The relevant Community legislative measure is:

- Directive 2002/46/EC of the European Parliament and of the Council on the approximation of the laws of the Member States relating to food supplements.

## TERMS OF REFERENCE AS PROVIDED BY THE COMMISSION

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority to provide a scientific opinion, based on its consideration of the safety and bioavailability of potassium molybdate added for nutritional purposes in food supplements.

## ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of Working Group A on Food Additives and Nutrient Sources of the ANS Panel for the preparation of this opinion: F. Aguilar, N. Bemrah, P. Galtier, J. Gilbert, S. Grilli, R. Guertler, G.E.N. Kass, C. Lambré, J.C. Larsen, J.-C. Leblanc, A. Mortensen, I. Pratt, I. Stankovic.

ASSESSMENT

## 1. Introduction

The present opinion deals with the safety of potassium molybdate, as a source of molybdenum, and with the bioavailability of molybdenum from this source, added for nutritional purposes in food supplements. The safety of molybdenum itself, in terms of the amounts that may be consumed, is outside the remit of this Panel.

## 2. Technical data

### 2.1. Chemistry

The molecular formula of potassium molybdate is $K_2MoO_4$, and its CAS Registry Number is 13446-49-6. The molecular mass is 238.14 g/mol. In this compound, molybdenum is present in its hexavalent form (molybdenum VI). This compound is characterized by an ionization constant of pKa = 4, corresponding to an almost complete dissociation at pH 7.

Other synonyms proposed by the petitioner are dipotassium molybdate, potassium orthomolybdate, potassium molybdenum oxide, dipotassium tetraoxomolybdate, molybdic acid, dipotassium salt.

Potassium molybdate is described as a white anhydrous powder, slightly deliquescent in air, which is soluble in water (124 g/100 ml at 25°C).

### 2.2. Specifications

The petitioner indicates that the purity is not less than 99.0%. Limits for impurities are: heavy metals ≦ 10 mg/kg, chloride ≤ 50 mg/kg, phosphates ≤ 200 mg/kg.

The Panel notes that according to Commission Regulation (EC) No 629/2008 (EC, 2008) the maximum levels of lead, mercury and cadmium in food supplements as sold should be 3 mg/kg, 0.1 mg/kg and 1 mg/kg, respectively.

### 2.3. Manufacturing process

Potassium molybdate ($K_2MoO_4$) is obtained by evaporating an aqueous solution of molybdenum trioxide and potassium hydroxide and heating at 100°C to yield the anhydrous salt.

### 2.4. Methods of analysis in food

For the determination of the source in food supplements, following extraction molybdenum may be measured by Atomic Absorption Spectroscopy (AAS) or Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

## 2.5. Reaction and fate in foods to which the source is added

The petitioner states that the compound is stable in food. However, no supporting data have been provided.

## 2.6. Case of need and proposed uses

Molybdenum is considered as a nutritionally essential trace element. It functions as an electron transport agent in the molybdenum-flavoprotein enzyme, xanthine oxidase. It is also a cofactor for aldehyde oxidase, aldehyde NADH-dehydrogenase, xanthine dehydrogenase and sulphite oxidase.

The petitioner stated that potassium molybdate is typically included in food supplements at 12-50 µg of potassium molybdate/day (2 tablets/day), corresponding to 5-20 µg of molybdenum/day, for adults. According to the petitioner, potassium molybdate is used as a powder, in tablets or in gelatin capsules.

## 2.7. Information on existing authorisations and evaluations

No data were provided on the source (potassium molybdate) itself. All information provided relates to molybdenum.

Both sodium and ammonium molybdates are already listed in the Annex of Commission Directive 2002/46/EC (EC, 2002), as substances which may be used in the manufacture of food supplements, and in the Commission Directive 2001/15/EC (EC, 2001), as substances that may be added for specific nutritional purposes in foods for particular nutritional uses.

The SCF (2000) considered that there were no adequate human data for establishing a tolerable upper intake level (UL). From toxicological data the critical effects of molybdenum in the rat and mouse appear to be effects on reproduction, and in particular foetal development. The pivotal animal study is a 9-week study in the rat showing a NOAEL of 0.9 mg molybdenum/kg bw/day (Fungwe *et al.*, 1990). An uncertainty factor (UF) of 100 was used. This provides an UL of approximately 0.01 mg/kg bw/day, equivalent to 0.6 mg/day for adults, which also covers pregnant and lactating women.

A further consideration is required in relation to ULs for children (Table 1), since an adverse effect on growth of young animals was seen in another study in rats, with a Lowest-Observed-Adverse-Effect-Level (LOAEL) of 2 mg/kg bw/day (Jeter and Davis, 1954). 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 SCF (SCF, 1993).

Table 1. **ULs for children (SCF, 2000)**

| 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 |

The EVM evaluation concluded that some human data suggested an increase in gout-like symptoms in populations consuming 1-15 mg of molybdenum/day, but that the majority of human data or the relevance of animal data was too uncertain to serve as the basis for an UL (EVM, 2003). In the face of such large uncertainties, but with some data suggesting adverse effects at lower levels, the EVM identified a guidance level (GL) for total intake, equal to intake from foods in the UK (0.230 mg/day). However, the EVM was not able to propose any guidance level of molybdenum from food and food supplements (EVM, 2003).

The US Food and Nutritional Board (FNB) and the US Environmental Protection Agency (EPA) recommended, respectively, a UL of 2 mg molybdenum/day (FNB, 2001) and an oral Reference Dose (RfD) of 0.005 mg/kg/day, or 0.35 mg/day for a 70 kg person (EPA, 2003).

The recommended daily allowance has been established at 45 µg/day (IOM 2001).

## 2.8. Exposure

The predominant form of molybdenum occurring in soil and natural waters is the molybdate anion ($MoO_4^{2-}$). Foodstuffs from above ground plant material, such as legumes, leafy vegetables and cauliflower, contain relatively high concentrations of molybdenum compared with food from tubers or animals.

Good food sources of molybdenum are sorghum, leafy vegetables, legumes (beans), grains (cereals, wheat germ), organ meats (liver, kidney), milk and eggs. Some 40% of molybdenum in cereals is lost on milling. Fruits, root vegetables and muscle meat are poor sources, whereas high concentrations have been found in shellfish. Soft tissue of fish contains about 1 mg molybdenum/kg, vascular plants 0.03-5 mg molybdenum/kg. Molybdenum levels in drinking water range from 0-68 µg/L, but usually do not exceed 10 µg/L (SCF, 2000).

Estimates of daily intake vary widely regionally depending on the soil type. For adults, the representative range of mean estimates of molybdenum intakes in different countries is 80-250 µg/day (SCF, 2000). High estimates of molybdenum intake in Europe range from 96 µg/day, in the Netherlands, to 500 µg/day, in Germany (SCF, 2000). Dietary molybdenum intake for children (1-17 years old) varies between 64-144 µg/day. Data from the French total diet study (Leblanc *et al.*, 2005) provided average intakes of molybdenum varying from 106 to 119 µg/day for children of 3-10 and 11-17 years old, respectively and high percentile intakes in the range of 199-208 µg/day.

According to the petitioner, molybdenum is intended to be used in food supplements to provide an intake of 5 to 20 µg molybdenum/day (2 tablets), corresponding to 12-50 µg of potassium molybdate/day for adults.

Information on molybdenum intake from food in European countries, anticipated exposure to molybdenum by using food supplements as proposed by the petitioner and ULs are summarised in Table 2.

Table 2. **Summary information on molybdenum intake and anticipated potential exposure to molybdenum from potassium molybdate.**

| Nutrient: Molybdenum | Amount (µg/day) | Average intake (µg/day) | High intake (µg/day) | References |
|---|---|---|---|---|
| Recommended Daily Allowance | 45 | | | IOM, 2001 |
| UL for adults | 600 | | | SCF, 2000 |
| UL for children (aged 1-17 years old) | 100-500 | | | SCF, 2000 |
| Intake range from food in Europe for adults | | 80-250 | 96-500 | SCF, 2000 |
| Intake range from food in Europe for children: | | | | |
| 1-3 years old | | 64-89 | | SCF, 2000 |
| 4-17 years old | | 80-144 | | |
| 3-10 years old | | | 199 | French total diet study (Leblanc *et al.*, 2005) |
| 11-17 years old | | | 208 | |
| Amount of molybdenum added to food supplements from potassium molybdate as indicated by petitioner (range) | 5-20 | | | Technical dossier, 2005 |
| | | | | |
| **Source: Potassium molybdate** | | | | |
| Total anticipated exposure to molybdenum from food supplement and food intake for adults[1] | | 100-270 | 116-520 | Calculation by the Panel |
| Total anticipated exposure to molybdenum from supplements and food intake range for children[2]: | | | | |
| 1-3 years old | | 84-109 | | Calculation by the Panel |
| 4-17 years old | | 100-164 | | |
| 3-10 years old | | | 219 | |
| 11-17 years old | | | 228 | |

[1] Calculation based on highest proposed supply use level of 20 µg molybdenum/day plus average dietary intake of 80-250 µg/day and high dietary intake of 96-500 µg/day for adults.

[2] Calculation based on highest proposed supply use level of 20 µg molybdenum/day plus average dietary intake range of 64-89 (1-3 years old) and 80-144 (4-17 years old) µg/day respectively, and high dietary intake of 199 (3-10 years old) and 208 (11-17 years old) µg/day for children.

In adult populations (over 18 years old) assuming a mean and a high level European dietary molybdenum intake (P95 or P97.5) in the range of 80-250 µg/day and 96-500 µg/day respectively, consumption of an additional food supplement containing 20 µg molybdenum or 50 µg potassium molybdate (the highest use level proposed by the petitioner) would result in a total anticipated daily molybdenum intake varying between 100 and 270 µg molybdenum/day in average level of dietary exposure and between 116 and 520 µg/day at the high level of dietary exposure, if no other food supplements containing molybdenum were taken (Table 2).

In children of 1-3 and 4-17 years old, assuming a mean dietary molybdenum intake in the range of 64-89 and 80-144 µg/day respectively, a consumption of an additional food supplement containing 20 µg molybdenum (the highest use level proposed by the petitioner) would result in a total anticipated daily molybdenum intake varying between 84-109 and 100-164 µg molybdenum/day, respectively. In children of 3-10 and 11-17 years old, assuming a high percentile dietary molybdenum intake of 199 µg/day and 208 µg/day respectively, a consumption of an additional food supplement containing 20 µg molybdenum (the highest use level proposed by the petitioner) would result in a total anticipated daily molybdenum intake of 219 and 228 µg molybdenum/day, respectively, at the high level of dietary exposure, if no other food supplements containing molybdenum were taken.

Based on the highest proposed supply use level of 50 µg of potassium molybdate/day for adults, an anticipated exposure to potassium from the supplement is 16 µg/day. The Panel considered this amount to be negligible compared to the average daily dietary intake of potassium, which ranges from 2.7-4 g/day (EFSA, 2005).

## 3. Biological and toxicological data

### 3.1. Bioavailability

Potassium molybdate like sodium molybdate is highly soluble in water. The petitioner states that there is no reason to suggest that its absorption profile will differ from that of other soluble molybdenum salts.

No data on bioavailability were provided by the petitioner on the source (potassium molybdate) itself.

#### *Animal studies*

The rate of gastrointestinal absorption of molybdenum depends on its chemical nature and the animal species (SCF, 2000). Ingested molybdenum(VI) but not molybdenum(IV), is readily absorbed from the duodenum and proximal jejunum. Water-soluble molybdates, thiomolybdates and oxothiomolybdates and molybdenum in herbage and green vegetables are absorbed to 75-97% by laboratory animals and ruminants. Silicates inhibit the absorption of dietary molybdates.

Female Sprague-Dawley rats aged 5 weeks were given 0.1, 1, or 10 mg molybdenum/L from sodium molybdate added to drinking water (Seaborn and Yang, 1993). Rats fed 0.1 mg molybdenum/L excreted more molybdenum in faeces than in urine, whereas rats fed 1 and 10 mg molybdenum/L water excreted more molybdenum in urine than in faeces, indicating that molybdenum absorption was not saturated as the urine level of molybdenum increased.

In rodents, molybdenum is distributed mainly to the liver, converted to molybdate with 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, molybdenum 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 due to limited absorption. Molybdenum is reabsorbed by the renal tubules but this reabsorption is reduced by S-containing and acid proteins. The reabsorbed molybdenum is deposited in the liver, lung, bone and skin (Patty's, 1981). Molybdenum-99 injected into dogs was concentrated in the liver, kidney, pancreas, pituitary, thyroid and adrenals but none appeared

in brain, white marrow or fat. The biological half-life varies from a few hours to several days in small laboratory animals and is related to the copper and sulphur metabolism (Patty's, 1981).

***Human data***

In humans, there is 40-50% absorption of water-soluble molybdenum compounds like molybdates and molybdenum present in herbage and green vegetables (WHO, 1996). The absorption from drinking water is likely to be similar to that from food. Twenty five percent of absorbed molybdenum appears rapidly in the blood, loosely bound to the erythrocytes and specifically bound to serum α2-macroglobulins (IDACE, 1995). Normal blood levels are 2-6 µg/L whole blood or 0.55 µg/L serum. Biological half-life of molybdenum may be up to several weeks in humans (Friberg and Lener, 1986).

In man, the highest levels appear in the kidney, liver and bone; raised levels appear also in adrenals and fat. 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 (SCF 2000).

The molybdenum content in human liver is 1.3-2.9 mg/kg dry weight, kidney 1.6 mg/kg dry weight, lung 0.15 mg/kg dry weight, brain and muscle 0.14 mg/kg dry weight, hair 0.07-0.16 mg/kg. Serum levels of molybdenum rise in liver functional defects, hepatitis, hepatic tumors and after certain drugs. Raised blood levels are seen in uremia, rheumatic disorders and cardiovascular disease (WHO, 1996).

## 3.2. Toxicological data

Potassium molybdate, like sodium molybdate, is highly soluble in water. The petitioner states that there is no reason to suggest that its toxicity profile will differ from that of other soluble molybdenum salts.

No data were provided on the source, potassium molybdate, itself.

### 3.2.1. Animal studies

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 in the normal range but the molybdenum content is considerably higher than the normal range.

The lethal dose for repeated oral administration is 60–333 mg/kg bw/day for soluble molybdenum compounds, including calcium or sodium molybdate, administered to rats, mouse, guinea pigs and rabbits (Fairhall *et al*., 1945; Arrington and Davis, 1953) but only about 3 mg sodium molybdate/kg bw/day for steers (Cook *et al*., 1966). Histological examinations of animals following acute doses generally show damage to the liver and kidney and sometimes to the adrenals and spleen.

Rabbits were exposed to oral doses of 0.025, 0.5, 5 and 50 mg molybdenum/kg bw/day in the diet as ammonium molybdate for 6 months. Body weight loss and fatty degeneration of the liver were noted at doses of 5 mg/kg bw/day and above, with the NOAEL being 0.5 mg/kg

bw/day. However, the weakness of the study was the uncertainty of the analytical method (Asmangulyan, 1965).

Rabbits of both sexes were exposed to oral doses of sodium molybdate in the diet equivalent to 1.8, 23, 46, 92, 184 mg molybdenum/kg bw/day for 4 months. The NOAEL was 23 mg/kg bw/day based on body weight loss, skeletal abnormalities and anemia (Arrington and Davis, 1953).

Guinea pigs of both sexes were treated for 8 weeks with doses of sodium molybdate in their diet rising by increments of 1000 to 8000 mg molybdenum/kg feed (1000 mg/kg feed corresponds to 75 mg molybdenum/kg bw/day). The LOAEL was 75 mg molybdenum/kg bw/day based on loss of copper, growth depression and achromotrichia. Guinea pigs appear to be a less sensitive species to large doses of molybdenum (Arthur, 1965).

The effects of sodium molybdate on the *in vivo* liver sulphation of xenobiotics in rats receiving a high oral dose of 1880 mg molybdate/kg have been examined in several studies (Boles and Klaassen, 1998a, 1998b, 2000). These studies have not been considered in the SCF opinion on the tolerable intake level of molybdenum (SCF, 2000). Results suggest that molybdate decreases the sulphation of dehydroepiandrosterone, harmol, alpha-naphtol and acetaminophen, by decreasing hepatic concentrations of the cosubstrate, 3'-phosphoadenosine-5'-phosphosulphate (PAPS).

### 3.2.2. Human data

Very little is known about specific effects of molybdenum compounds on human health and there are no well designed chronic studies in man which can be used for risk assessment.

The only available studies refer to putative effects of molybdenum in foods, drinking water or to data obtained by using stable isotopes of molybdenum as tracers. In an area in Armenia, where the population is exposed to a high dietary intake of molybdenum, due to geophysical soil levels of 77 mg molybdenum/kg and 39 mg copper/kg, aching joints, elevated concentrations of uric acid in the blood and urine, increased blood molybdenum-containing xanthine oxidase (XO) activity and gout-like symptoms have been reported (Kovalsky *et al.*, 1961).

Serum uric acid levels were compared in individuals of two Colorado cities with low (2-50 µg/L) and high (≥ 220 µg/L) molybdenum levels in their drinking water (Chappel *et al.*, 1979). Among subjects consuming water containing up to 50 µg molybdenum/L, plasma molybdenum levels were within the normal range and no adverse biochemical or systemic effects were noted. The higher molybdenum intake was associated with higher serum ceruloplasmin and lower serum uric acid.

In a study on four young men fed dietary doses of metallic molybdenum varying from 0.022-1.490 mg/day for 24 days, no adverse effects were noted (Turnlund *et al.*, 1995).

### 3.2.3. Genotoxicity and carcinogenicity

Conflicting information is available on the genotoxicity of molybdates. Ammonium heptamolybdate was mutagenic in *Escherichia coli* strains and positive in the *Bacillus subtilis* rec-assay (Nishioka, 1975). On the other hand, soluble molybdates were not mutagenic in *Escherichia coli* (Venitt and Levy, 1974), *Bacillus subtilis* (Kada *et al.*, 1980) and *Salmonella typhimurium,* with or without metabolic activation (Zeiger *et al.*, 1992; NTP, 1997).

Ammonium and sodium molybdate were neither mutagenic nor recombinogenic in *Saccharomyces cerevisiae* reverse mutation and gene conversion assays (Singh, 1983).

Ammonium molybdate (10 μM, 24 h) induced chromosome aberrations and sister-chromatid exchanges (SCEs) in human lymphocytes *in vitro* (Bobyleva *et al.*, 1991). Molybdenum trioxide (8 and 15 mg/kg bw), but not ammonium molybdate (2.5 and 7.5 mg/kg bw), induced chromosome damage in mouse bone marrow (Chopikashvili *et al.*, 1991). The same authors reported dominant lethal mutations and decreased survival in Drosophila exposed to 2–4 g of ammonium molybdate per kg of feed. The Panel was not able to obtain more detailed information from these papers, published in Russian.

As reported by Titenko-Holland *et al.* (1998), both ammonium molybdate (0.1–2 mM) and sodium molybdate (0.1-5 mM) increased the incidence of micronucleated cells in cytokinesis-blocked cultured human lymphocytes of a female and a male donor. The largest effect (about a four-fold increase) was elicited by ammonium molybdate.

Forty-eight hour treatments with both molybdate salts were associated with a decreased viability (up to 70-80% compared to negative control) and reduced cell proliferation, as indicated by the lower replicative index of treated cultures. The determination of centromere content in micronuclei by fluorescence *in situ* hybridization with centromeric probe indicated that the increase in micronuclei observed in treated cultures was in all cases almost totally due to the induction of chromosome loss. Conversely, no consistent (more than two-fold) or dose-related increase of micronuclei, without centromere signal markers of clastogenic events, was observed.

Two intraperitoneal doses of sodium molybdate, 200 and 400 mg/kg bw/day, were assessed in the bone marrow micronucleus (MN) assay in mice (injections 24 and 48 hours prior to euthanasia). A modest increase in the overall incidence of MN frequency in polychromatic erythrocytes of treated animals was observed. However, no definitive conclusion on the biological significance of this finding can be drawn, due to the lack of information on data heterogeneity and/or individual animal results. The same treatment protocol was used to analyse dominant lethality. A dose-dependent increase (up to three-fold) in post-implantation loss was observed in females mated at the first week after treatment of males. These data may suggest that sodium molybdate induces dominant lethality at the post-meiotic stage of spermatogenesis. However, these findings need to be interpreted with caution, due to some inconsistencies in test results (e.g. the variable spontaneous incidence of early losses among different study groups).

The Panel considers that the results of this study (Titenko-Holland *et al.*, 1998) suggest that ammonium molybdate and sodium molybdate induce chromosome loss in human lymphocytes *in vitro*, and provided limited evidence of genotoxicity *in vivo* in mouse somatic and germ cells. This information was not included in the SCF opinion on the tolerable intake level of molybdenum (SCF, 2000).

There are no relevant studies on the carcinogenicity of molybdenum in animals or humans. With the exception of dust exposure, molybdates are not on the Maximale Arbeitsplatz-Konzentration (MAK) list (2008), EPA list or ACGIH list (Vyskocil and Viau, 1999).

### 3.2.4. Reproduction and teratogenicity

Mice were given a daily single dose of sodium molybdate, corresponding to 10 mg molybdenum/L (1.5 mg molybdenum/kg bw), in their drinking water for 6 months or about 3 generations. Excess pup deaths (15/238) in the $F_1$ generation and 7/242 pup deaths plus five

dead litters and one maternal death in the $F_2$ generation and infertility were noted. This would correspond to a LOAEL of 1.5 mg molybdenum/kg bw/day (Schroeder and Mitchener, 1971).

In a 13-week study, Long-Evans rats were given dietary doses of sodium molybdate corresponding to 20, 80, 140, 700 mg molybdenum/kg feed (calculated to represent approximately 2, 8, 14, 70 mg molybdenum/kg bw/day) and either 5 or 20 mg copper/kg bw additionally. Growth depression was observed at the lowest dose in males. 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 molybdenum 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-week study in Sprague-Dawley rats on the effects of molybdenum supplementation on estrus activity, fertility and foetal development, 5 groups, each of 21 female weaning rats, were given a basic diet containing 0.025 mg molybdenum/kg diet as well as 6.3 mg copper/kg diet for 6 weeks, and additionally in their drinking water doses of 0, 5, 10, 50 and 100 mg molybdenum/L as sodium molybdate for 3 weeks until the 21$^{st}$ day of gestation (Fungwe *et al.*, 1990). Six animals in each group were used 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 molybdenum intakes were 0, 0.64, 1.12, 5.81 and 11.56 mg molybdenum/rat/week, corresponding to 0, 0.91, 1.6, 8.3 and 16.7 mg molybdenum/kg bw/day assuming a 100 g/bw as indicated by the SCF (2000). There was no effect on fertility, food and water consumption. Oestrus cycle was prolonged by 1.6 mg molybdenum/kg bw/day and higher supplementations. Gestational weight, litter size and foetal weights were lower than controls for the groups fed 1.6 mg molybdenum/kg bw/day and higher doses. Histopathology showed delayed development of foetal structures, delayed esophageal development, delayed transfer of foetal hematopoeisis from liver to bone marrow, and delayed myelination of the spinal cord at doses of 1.6 mg molybdenum/kg bw/day and above. Fetal resorption was increased at doses of 1.6 mg molybdenum/kg bw/day and higher. Sulphite oxidase, xanthine dehydrogenase and XO activities increased with molybdenum supplementation but less in pregnant animals at dose levels of 1.6 mg molybdenum/kg bw/day and above. The NOAEL was 0.9 mg molybdenum/kg bw/day.

Four pregnant Cheviot ewes were given in their feed an extra 50 mg molybdenum/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).

## 4. Discussion

Sodium molybdate and ammonium molybdate are already listed in Annex II of the Commission Directive 2002/46/EC, as substances which may be used as a source of molybdenum in food supplements. The petitioner argues for the addition of the equally soluble potassium molybdate, which is also dissociated in solution in water, to provide a source of molybdenum supplied as a nutrient in food supplements.

The Panel considers that due to its ionisation properties (pKa = 4), the non-ionised form of potassium molybdate should be absorbed by a diffusion process in the stomach. Potassium molybdate should also dissociate into its constituents, potassium and molybdate ions, in the small intestine. Accordingly, molybdenum from potassium molybdate should be bioavailable and absorbed in the same manner as molybdenum from other soluble molybdates.

No data on toxicity were provided on the source (potassium molybdate) itself.

The SCF established an UL for molybdenum of approximately 0.01 mg/kg bw/day, equivalent to 0.6 mg /day for adults, which also covers pregnant and lactating women, with proportionate ULs for children (0.1 to 0.5 mg/person/day) on a body weight basis (SCF, 2000). According to the petitioner, potassium molybdate is intended to be used in food supplements to provide an intake of 5-20 µg molybdenum/day, equivalent to 12-50 µg of potassium molybdate/day, which will not exceed the UL of 0.6 mg molybdenum/day established by the SCF.

The Panel notes that since the SCF adopted its opinion on molybdenum, new *in vitro* and *in vivo* genotoxicity data have been made available on molybdenum that might need further investigation.

Estimates of dietary intake of molybdenum vary widely regionally depending on the soil type. For adults, the representative range of mean estimates of molybdenum intakes in different countries is 80-250 µg/day (SCF, 2000). High estimates of molybdenum intake in Europe range from 96 µg/day, in the Netherlands, to 500 µg/day, in Germany (SCF, 2000). Based on the information provided by the petitioner, and assuming an average dietary molybdenum intake in the range of 80-250 µg/day, the Panel estimated that consumption of an additional food supplement providing 20 µg molybdenum/day (highest proposed use level) would result in a total anticipated dietary exposure of between 100 and 270 µg molybdenum/day in an adult at the average level of dietary average exposure. When the high dietary intake range for the European population of 96-500 is considered, the consumption of an additional food supplement providing 20 µg/day would result in a total anticipated exposure of between 116 and 520 µg molybdenum/day. The Panel notes that this molybdenum exposure would not exceed the UL of 0.6 mg/day as established by the SCF in 2000 for adults.

The Panel notes that the use levels proposed by the petitioner (between 5 and 20 µg/day) would not exceed the ULs of 0.1 to 0.5 mg molybdenum/day defined by the SCF for children (1-17 years old). However, given the wide range of UL values established by the SCF based on age body weights, the Panel considers that in some cases these ULs could be exceeded when molybdenum intake from food is taken into account together with food supplementation.

Assuming a mean dietary intake range between 64-89 and 80-144 µg molybdenum/day for children between 1-3 and 4-17 years old, respectively, in addition to a daily consumption of an additional food supplement containing 20 µg molybdenum (the highest use level proposed by the petitioner), the Panel estimates that the use of potassium molybdate in food supplements could result in a total anticipated exposure of between 84-109 µg and 100-164 µg molybdenum/day, respectively. The Panel notes that this molybdenum exposure exceeds the UL of 0.1 mg/person/day as established by the SCF in 2000 for children between 1 and 3 years of age.

Assuming a high percentile dietary molybdenum intake of 199 µg/day and 208 µg/day for age groups between 3-10 and 11-17 years old, respectively, the Panel estimates that a consumption of an additional food supplement containing 20 µg molybdenum (the highest use level proposed by the petitioner) would result in a total anticipated daily molybdenum intake of 219 and 228 µg molybdenum/day respectively, at the high level of dietary exposure. The Panel notes that this molybdenum exposure exceeds the UL of 0.2 mg/person/day as established by the SCF in 2000 for children between 4 and 6 years of age.

Based on the highest proposed use level of 50 µg of potassium molybdate/day for adults, an anticipated exposure to potassium from the food supplements is 16 µg/day. The Panel

considered this amount to be negligible compared to the average daily dietary intake of potassium, which ranges from 2.7-4 g/day.

## CONCLUSIONS

The present opinion deals with the safety of potassium molybdate, as a source of molybdenum, and with the bioavailability of molybdenum from this source, added for nutritional purposes in food supplements. The safety of molybdenum itself, in terms of the amounts that may be consumed, is outside the remit of this Panel.

The Panel considers that the non-ionised form of potassium molybdate is expected to be absorbed in the stomach. Potassium molybdate should also dissociate into its constituents, potassium and molybdate ions, in the small intestine. Accordingly, molybdenum from potassium molybdate should be bioavailable and absorbed in the same manner as molybdenum from other soluble molybdates.

Based on the highest proposed use level of 50 µg of potassium molybdate/day for adults, the anticipated exposure to potassium from the food supplements is 16 µg/day. The Panel considers this amount to be negligible compared to the average daily dietary intake of potassium, which ranges from 2.7-4 g/day (SCF, 2003).

The anticipated exposure to molybdenum from the use of potassium molybdate for adults, at the highest proposed use level of 20 µg/day is below the UL of 0.6 mg/day. The Panel notes however that when dietary intakes of molybdenum are taken into consideration, the UL of 0.1 mg/day for children (1-3 years old) or 0.2 mg/day for children (4-6 years old) is likely to be exceeded at average and high percentile intake level, respectively.

The Panel concludes that the use of potassium molybdate, as a source of molybdenum, added for nutritional purposes in food supplements at the proposed use level, is of no safety concern provided the applicable UL for molybdenum established by the SCF is not exceeded .

The Panel notes that since the SCF adopted its opinion on molybdenum, new toxicological data have been made available on *in vitro* and *in vivo* genotoxicity of molybdenum that might need further investigation.

## DOCUMENTATION PROVIDED TO EFSA

1. Dossier on Potassium Molybdate added for nutritional purposes to food supplements. May 2005. Submitted by Health Food Manufacturers' Association.

## REFERENCES

Arrington LR and Davis GK, 1953. Molybdenum toxicity in the rabbit. Journal of Nutrition 51, 295–304.

Arthur D, 1965. Interrelationships of molybdenum and copper in the diet of the guinea pig. Journal of Nutrition 51, 295-304.

Asmangulyan A, 1965. Determination of the maximum permissible concentration of molybdenum in open bodies of water. Gigiena i sanitariia 30, 4-6.

Bobyleva LA, Chopikashvili LV, Alyokhina NI, Zasukhina GD, 1991. Chromosome aberrations and sister chromatid exchanges in high risk groups of workers exposed to heavy metals. Cytology and Genetics (Russia) 25(3), 18–23.

Boles JW, Klaassen CD, 1998a. Effect of molybdate on the sulfation of harmol and alpha-naphtol. Toxicology 127, 121-127.

Boles JW, Klaassen CD, 1998b. Effect of molybdate and pentachlorophenol on the sulfation of dehydroepiandrosterone. Toxicology and Applied Pharmacology 151, 105-109.

Boles JW, Klaassen CD, 2000. Effect of molybdate and pentachlorophenol on the sulfation of acetaminophen. Toxicology 146, 23-35.

Chopikashvili LV, Bobyuleva LA, Zolotareva GN, 1991: Genotoxicity of molybdenum and its derivatives in *Drosophila* and mammals. Cytology and Genetics (Russia) 25(5), 45–49.

Cook GA, Lesperance AL, Bohman VR, Jensen EH, 1966. Interrelationship of molybdenum and certain factors to the development of the molybdenum toxicity syndrome. Journal of Animal Science 25, 96–101.

EC (European Commission), 2001. Commission Directive 2001/15/EC of 15 February 2001 on substances that may be added for specific nutritional purposes in foods for particular nutritional uses. Official Journal of the European Communities L 52, 22.02.2001, p.19.

EC (European Commission), 2002. Commission Directive 2002/46/EC of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements. Official Journal of the European Union, Official Journal of the European Communities L 183, 12.7.2002 p.51.

EC (European Commission), 2008. Commission Regulation (EC) No 629/2008 of 2 July 2008 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuff. Official Journal of the European Communities L 173, 3.07.2008.

EFSA (European Food Safety Authority), 2005. Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to the Tolerable Upper Intake Level of Potassium. The EFSA Journal 193, 1-19.

EPA (Environmental Protection Agency), 2003. Molybdenum (CASRN 7439-98-7) US Environmental Protection Agency Integrated Risk Information System. Last revision 28 October 2003.

EVM (Expert Group on Vitamins and Minerals), 2003. Part 3: trace elements. In Safe Upper Levels for Vitamins and Minerals: Report of the Expert Group on Vitamins and Minerals. 172-179. 2003. Food Standards Agency (FSA), London, England

Fairhall L, Dunn RC, Sharpless NE Pritchard EA, 1945. The Toxicity of Molybdenum. U.S. Public Health Bull. No. 293. (Cited in Friberg *et al.*, 1975; Stokinger, 1981; U.S. EPA, 1990).

Friberg L and Lener J, 1986. Molybenum. In: Handbook on the Toxicology of Metals. 2nd ed. L Friberg, GF Nordberg and VB Vouk, eds. Elsevier/North-Holland Biomedical Press, New York. pp. 446-461.

Fungwe F, Buddingh DS, Demick CD, Lox MT, Yang MT, Yang SP, 1990. The role of dietary molybdenum on estrous activity, fertility, reproduction and molybdenum and copper enzyme activities of female rats. Nutrition Research 10, 515–524.

IDACE (Association of Dietetic Food Industries of the EU), 1995. Request to the EU Commission on Molybdates (CS/ADD/NUT/2).

IOM (Institute of Medicine), 2001. Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc. National Academic Press, Washington, DC.

Jeter M, Davis GK, 1954. The effect of dietary molybdenum upon growth, hemoglobin, reproduction and lactation of rats. Journal of Nutrition 54, 215-220.

Kada T, Hirano K, Shirasu Y, 1980. Screening of environmental chemical mutagens by the rec-assay system with *Bacillus subtilis.* In de Serres FJ, Hollaender A (eds): ‘‘Chemical Mutagens: Principles and Methods for Their Detection, Vol. 6.’’ New York: Plenum Press, pp 149–173.

Kovalsky VV, Yarovaya GA, Shmavonyan DM. 1961. Changes of purine metabolism in man and animals under conditions of molybdenum biogeochemical provinces. Zhurnal obshcheĭ biologii 22, 179-191.

Leblanc JC, Guerin T, Noel L, Calamassi-Tran G, Volatier JL, Verger P, 2005. Dietary exposure estimates of 18 elements from the 1st French Total Diet Study. Food Additives and Contaminants 22, 624-641.

Mills CF and Fell BF, 1960. Demyelination in lambs born of ewes maintained on high intakes of sulphate and molybdate. Nature 185, 20-22.

NTP (National Toxicology Program), 1997. Molybdenum trioxide. NTP TR 462, NIH Pub. No. 95–3378.

Nishioka H, 1975. Mutagenic activities of metal compounds in bacteria. Mutation Research 31, 185-189.

Patty’s Industrial Hygiene and Toxicology, 1981. 3rd edition, Wiley Interscience Publ.

SCF (Scientific Committee for Food), 1993. 31st series of reports of the Scientific Committee for Food. Nutrient and energy intakes for the European Community. Commission of the European Communities, Luxembourg

SCF (Scientific Committee on Food), 2000. Opinion of the Scientific Committee on Food on The Tolerable Upper Intake Level of Molybdenum. SCF/CS/NUT/UPPLEV/22 November 2000. Commission of the European Communities, Luxembourg.

Schroeder HA, Mitchener M, 1971. Toxic effects of trace elements on the reproduction of mice and rats. Archives of environmental health 23, 102-106.

Seaborn CD, Yang SP, 1993. Effect of molybdenum supplementation on N-nitroso-N-methylurea-induced mammary carcinogenesis and molybdenum excretion in rats. Biological trace element research 39, 245-256.

Singh I, 1983. Induction of reverse mutation and mitotic gene conversion by some metal compounds in *Saccharomyces cerevisiae*. Mutation research 117 (1-2), 149-52.

Titenko-Holland N, Shao JS, Zhang LP, Xi LQ, Ngo HL, Shang N, Smith, MT, 1998. Studies on the genotoxicity of molybdenum salts in human cells *in vitro* and in mice *in vivo*. Environmental and molecular mutagenesis 32, 251-259.

Turnlund JE, Keyes WR, Peiffer GL, 1995. Molybdenum absorption, excretion, and retention studied with stable isotopes in young men at five intakes of dietary molybdenum. The American journal of clinical nutrition 62, 790–7796.

Venitt S, Levy LS, 1974: Mutagenicity of chromates in bacteria and its relevance to chromate carcinogenesis. Nature 250, 493–495.

Vyskocil A and Viau C, 1999. Assessment of molybdenum toxicity in humans. Journal of applied toxicology 19, 185-92.

WHO (World Health Organization), 1996. Trace Elements in Human Nutrition and Health. Geneva. World Health Organization. Prepared in Collaboration with the FAO of the UN and the IAEA.

Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K, 1992. *Salmonella* mutagenicity tests. V. Results from the testing of 311 chemicals. Environmental and molecular mutagenesis 19 (Suppl 21), 2–141.

## GLOSSARY / ABBREVIATIONS

| | |
|---|---|
| AAS | Atomic Absorption Spectroscopy |
| ANS Panel | Scientific Panel on Food Additives and Nutrient Sources added to Food |
| CAS | Chemical Abstracts Service |
| EC | European Commission |
| EFSA | European Food Safety Authority |
| EPA | Environmental Protection Agency |
| EVM | UK Expert Group on Vitamins and Minerals |
| FNB | US Food and Nutrition Board |
| ICP-MS | Inductively Coupled Plasma Mass Spectrometry |
| IOM | Institute of Medicine |
| LOAEL | Lowest-Observed-Adverse-Effect-Level |
| MN | Micronucleus |
| NOAEL | No-Observable-Adverse-Effect-Level |
| RfD | Reference Dose |
| SCF | Scientific Committee on Food |
| UL | Tolerable Upper Intake Level |
| WHO | World Health Organization |