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Safety and efficacy of a molybdenum compound (E7) sodium molybdate dihydrate as feed additive for sheep based on a dossier submitted by Trouw Nutrition International B.V.

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Abstract

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on safety and efficacy of sodium molybdate dihydrate for sheep, based on a dossier submitted for the re-evaluation of the additive. The additive is currently authorised in the EU for all animal species as 'Nutritional additive' – 'Compounds of trace elements'. Taking the optimal Cu:Mo ratio of 3–10, and the highest total copper level authorised in complete feeds for sheep (15 mg/kg), the FEEDAP Panel concluded that 2.5 mg total Mo/kg complete feed is safe for sheep. Considering (i) a safe intake of 0.6 mg Mo/day, (ii) the estimate average intake figure from food in Europe (generally less than 100 μg/day), (iii) the contribution of foods of animal origin to the total molybdenum intake (estimated to be up to 22 %), and (iv) that molybdenum would not accumulate in edible tissues/products of sheep fed molybdenum supplemented diets up to the upper safe level, the FEEDAP Panel concluded that the use of sodium molybdate as a additive in sheep at 2.5 mg total Mo/kg complete feed is safe for consumers. The additive under assessment feed poses no risk by inhalation to users; it is a skin and eye irritant, but it is not considered as a skin sensitiser. Sodium molybdate used up to 2.5 mg Mo/kg complete sheep feed poses no concerns for the safety for the environment. The FEEDAP Panel recognises that molybdenum does not need to be added to diets to cover the nutritional needs of molybdenum of sheep. Molybdenum supplementation in sheep feed is considered effective in order to guarantee an adequate balance with copper, when the Cu:Mo ratio in the diet is in the range 3–10.

Keywords: nutritional additive, compounds of trace elements, molybdenum, sodium molybdate dihydrate, safety, efficacy, sheep

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Summary

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on safety and efficacy of sodium molybdate dihydrate for sheep. The additive is currently authorised in the European Union (EU) under the element Molybdenum-Mo (E7) for all animal species; the compound is included in the EU Register of Feed Additives under the category 'Nutritional additives' and the functional group 'Compounds of trace elements'.

Molybdenum toxicity in farm animals is manifested as antagonism of absorption and/or biological activity of copper, and is greatly enhanced by high sulfur content in the diet. Ruminants, including sheep, are highly susceptible to molybdenum excess, which may induce a clinically severe copper deficiency (molybdenosis). Conversely, low molybdenum in the diet is expected to enhance copper toxicity, if the intake of copper is high. The FEEDAP Panel considered therefore not possible to establish an absolute figure for a dietary molybdenum concentration which is equally safe for sheep and effective in preventing copper toxicity. Considering that (i) the key parameter to ensure the safety of molybdenum supplementation is the optimal Cu:Mo ratio, which in sheep is in the range of 3–10 and (ii) the highest total copper level authorised in complete feeds for sheep is 15 mg/kg, the FEEDAP Panel concluded, that 2.5 mg total Mo/kg complete feed is safe for sheep.

Toxicokinetic data in laboratory rodents and farm animals (including sheep), however incomplete, uniformly indicate that molybdenum would not accumulate in edible tissues or products of sheep fed molybdenum supplemented diets up to the upper maximum level of 2.5 mg/kg. The FEEDAP Panel considered that the available data support an upper intake tolerable level (UL) of 0.01 mg/kg body weight (bw) for molybdenum based on the no observed adverse effect level (NOAEL) for female reproductive toxicity and developmental toxicity of 0.9 mg/kg bw per day and the application of a 100-safety factor. The UL would result in a safe intake of 0.6 mg/day in a 60-kg individual; this intake is largely higher than the estimate average intake figure from food in Europe (generally less than 100 μg/day). Molybdenum is ubiquitous in foods, surveys in the EU countries provide average intake figures generally lower than 100 μg/day, whereas offals (liver and kidney) are relatively rich sources of molybdenum, the contribution of foods of animal origin to the total molybdenum intake has been estimated to be up to 22%. Molybdenum would not accumulate in edible tissues or products of sheep fed molybdenum supplemented diets up to the upper maximum level of 2.5 mg/kg. Therefore, the FEEDAP Panel considered that the use of sodium molybdate as a feed additive in sheep at 2.5 mg Mo/kg complete feed is safe for consumers.

Molybdenum is a potential respiratory toxicant; the available data indicate that the use of the sodium molybdate under evaluation in animal nutrition poses no risk by inhalation to users. The additive is a skin and eye irritant, but it is not considered as a skin sensitiser.

The use of sodium molybdate as a feed additive in sheep up to maximum of 2.5 mg of Mo/kg complete feed poses no concerns for the safety for the environment.

The FEEDAP Panel recognises that molybdenum does not need to be added to diets to cover the nutritional needs of molybdenum of sheep. Molybdenum supplementation in sheep feed is considered effective in order to guarantee an adequate balance with copper, when the Cu:Mo ratio in the diet is in the range 3–10.

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003 establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 10(2) of that Regulation also specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, at the latest one year before the expiry date of the authorisation given pursuant to Directive 70/524/EEC for additives with a limited authorisation period, and within a maximum of seven years after the entry into force of this Regulation for additives authorised without a time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from Trouw Nutrition International B.V. for re-evaluation of the molybdenum-containing additive, sodium molybdate dihydrate, when used as a feed additive for sheep[1] (category: Nutritional additives; functional group: compounds of trace elements).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 10(2) (re-evaluation of an authorised feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 25 January 2016.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the product sodium molybdate dihydrate, when used under the proposed conditions of use (see Section 3.1.3).

1.2. Additional information

The additive 'Sodium molybdate' had been authorised in the European Union (EU) under the element Molybdenum-Mo (E7) for all animal species 'Without a time limit' (Council Directive 70/524/EEC concerning additives in feedingstuffs – List of authorised additives in feedingstuffs (2004/C 50/01). Following the provisions of Article 10(1) of Regulation (EC) No 1831/2003 the compound was included in the EU Register of Feed Additives under the category 'Nutritional additives' and the functional group 'Compounds of trace elements'.

The Scientific Committee on Food (SCF) of the European Commission published in the year 2000 an opinion on the tolerable upper intake levels of molybdenum (European Commission, 2000). The EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS Panel) delivered an opinion on potassium molybdate as a source of molybdenum added for nutritional purposes to food supplements (EFSA, 2009). The EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA Panel) delivered an opinion on dietary reference values for molybdenum (EFSA NDA Panel, 2013).

According to Regulation (EC) no 1170/2009[2], *Molybdenum* is listed as mineral which may be used in the manufacture of food supplements (Annex I); the following molybdenum compounds are authorised for use in the manufacture of food supplements: ammonium molybdate (molybdenum (VI)), potassium molybdate (molybdenum (VI)) and sodium molybdate (molybdenum (VI)) (Annex II); the following molybdenum compounds are authorised as mineral substances which may be added to foods: ammonium molybdate (molybdenum (VI)) and sodium molybdate (molybdenum (VI)) (Annex III).

The following molybdenum compounds may be added for specific nutritional purposes in foods for particular nutritional uses (Commission Regulation (EC) No 953/2009)[3]: ammonium molybdate and sodium molybdate.

[1] Initially, the application was submitted for 'All animal species'. During the course of the evaluation, the applicant requested to the EC a partial withdrawal of the target species, limiting the application only for sheep. Subsequently the EC informed EFSA on the request (e-mail dated 22.2.2017).

[2] Commission Regulation (EC) No 1170/2009 of 30 November 2009 amending Directive 2002/46/EC of the European Parliament and of Council and Regulation (EC) No 1925/2006 of the European Parliament and of the Council as regards the lists of vitamin and minerals and their forms that can be added to foods, including food supplements. OJ L 314, 1.12.2009, p. 36.

[3] Commission Regulation (EC) No 953/2009 of 13 October 2009 on substances that may be added for specific nutritional purposes in foods for particular nutritional uses. OJ L 269, 14.10.2009, p. 9.

The following types of fertilisers containing molybdenum and described as 'Fertilisers containing only one micro-nutrient' are listed in Annex I of Regulation (EC) No 2003/2003 of the European Parliament and of the Council[4] as: (a) sodium molybdate (chemically obtained product containing sodium molybdate as its essential ingredient), (b) ammonium molybdate (chemically obtained product containing ammonium molybdate as its essential ingredient), (c) molybdenum-based fertiliser Product obtained by mixing types (a) and (b)), and (d) molybdenum-based fertiliser solution (product obtained by dissolving types '(a)' and/or one of the type '(b)' in water).

According to the Annex to Regulation (EC) No 432/2012[5], molybdenum contributes to normal sulfur amino acid metabolism, the claim may be used only for food which is at least a source of molybdenum as referred to in the claim SOURCE OF [NAME OF VITAMIN/S] AND/OR [NAME OF MINERAL/S] as listed in the Annex to Regulation (EC) No 1924/2006[6].

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[7] in support of the authorisation request for the use of sodium molybdate dihydrate as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008[8] and the applicable EFSA guidance documents.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers, other scientific reports and experts' elicitation knowledge, to deliver the present output.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the product sodium molybdate dihydrate in animal feed. The Executive Summary of the EURL report can be found in the Annex A.[9]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of sodium molybdate dihydrate is in line with the principles laid down in Regulation (EC) No 429/2008[8] and the relevant guidance documents: Guidance on nutritional additives (EFSA FEEDAP Panel, 2012a), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008a), Guidance for the preparation of dossiers for the re-evaluation of certain additives already authorised under Directive 70/524/EEC (EFSA, 2008b), Guidance for the preparation of dossiers for additives already authorised for use in food (EFSA FEEDAP Panel, 2012b), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012c) and the Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012d).

3. Assessment

The trace element molybdenum is an essential component of certain enzymes that catalyse redox reactions and contain, in addition to molybdenum, other prosthetic groups such as flavin adenine dinucleotide or haem. In animals, such enzymes include xanthine, aldehyde and sulfite oxidases involved in the metabolism of aromatic aldehydes and nicotinic acid and the catabolism of sulfur-containing amino acids and heterocyclic compounds, including purines, pyrimidines, pteridins and pyridines. In addition, molybdenum functions as an electron carrier in nitrate reductase and nitrogenase that catalyse the

[4] Regulation (EC) No 2003/2003 of the European Parliament and of the Council of 13 October 2003 relating to fertilisers. OJ L 304, 21.11.2003, p. 1.

[5] Commission Regulation (EC) No 432/2012 of 16 May 2012 establishing a list of permitted health claims made on foods, other than those referring to the reduction of disease risk and to children's development and health. OJ L 136, 25.5.2012, p.1.

[6] Regulation (EC) No 1924/2006 of the European Parliament and of the council of 20 December 2006 on nutrition and health claims made for food. OJ L 404, 30.12.2006, p. 9.

[7] FEED dossier reference: FAD-2010-0256.

[8] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

[9] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2010-0256-sodium_molybdate.pdf

reduction of nitrogen and nitrate (Spears, 1999; EFSA NDA Panel, 2013). The crystal structure of xanthine oxidase in bovine milk, including the molybdopterin-binding domain, was established by Enroth et al. (2000).

Even though molybdenum has been demonstrated to be essential in experimental studies with animals fed highly purified diets (Anke et al., 1985a,b), the EFSA Panel on dietetic products, nutrition and allergies (NDA Panel) in 2013 concluded that in humans molybdenum deficiency has not been observed and there are no biomarkers of molybdenum status (EFSA NDA Panel, 2013). A distinct molybdenum deficiency syndrome has not been observed either in animals when subjected to molybdenum restriction. Low dietary molybdenum leads to reduced activity of molybdoenzymes, low urinary and serum uric acid concentrations and excessive xanthine excretion. Besides these biochemical changes, spontaneously occurring adverse health effects directly related to molybdenum deficiency have not been described in farm animals (Spears, 1999; NRC, 2007) or humans (EFSA NDA Panel, 2013) under practical conditions.

Quantitative information about requirements of molybdenum in animals or humans is limited and inconsistent. Chiang et al. (1989) suggested that in adult humans the intake of 475 μg Mo/day (about 8 μg/kg body weight (bw)) normalised the biomarkers that indicated suboptimal molybdenum intake. This value is in the same range as the findings by Wang et al. (1989) in female rats: reduced activities of Mo-depending enzymes (xanthine dehydrogenase; xanthine oxidase) in the liver and intestinal mucosa were induced by diets containing 25 μg, but not with > 50 μg Mo/kg feed (corresponding to approximately 1.3 and 2.5 μg Mo/kg bw per day). Much lower basal requirements in humans were indicated by WHO (1996) at approximately 25 μg Mo/day, corresponding to about 0.4 μg Mo/kg bw in a 60-kg individual. These values are in agreement with data summarised by Tallkvist and Oskarsson (2015). The EFSA NDA Panel has defined an adequate intake (AI) of 65 μg/day for adults, whereas a single study suggested that the basal requirements could be 22 μg/day (EFSA NDA Panel, 2013).

With the exception of ruminants, data on farm animals are also limited. Although some early studies had suggested that in chicken a number of adverse symptoms like loss of feathers, disorders of ossification of long bones and changes in joint cartilage can be reversed by the addition of 0.2–2.5 mg Mo/kg feed, in reviewing such studies, Tallkvist and Oskarsson (2015) threw some doubts about the real effects of molybdenum supplementation, which require further experimental verification. Nowadays it is generally accepted that deficiency of molybdenum occurs rarely, if ever, in farm animals in field conditions, also because the basal diets are usually adequate in satisfying the basal requirements.

In ruminants (and particularly in sheep), low concentrations of molybdenum in the diet are associated with high risk of copper toxicity and the protective effect of molybdenum supplementation towards copper toxicity in ruminants exposed to high environmental copper concentrations is well established since decades (NRC, 1985; Suttle, 2010). On the other hand, excess of molybdenum in the diet leads to secondary copper deficiency in ruminants (Suttle, 2010). The effect of molybdenum on copper metabolism, nearly exclusive in ruminants on practical conditions, is due to the formation in the rumen (in the presence of sulfur adequate concentrations) of thiomolybdates that sequester copper. This complex non-competitive interaction Cu-S-Mo makes that copper requirements in ruminants cannot be established without considering the molybdenum and sulfur concentrations. In the same way, it is impossible to give clear 'requirement' values of molybdenum for ruminants under field conditions, since the copper and sulfur content of feed are variable (Suttle, 2010; Tallkvist and Oskarsson, 2015). Based on this, Suttle (2010) proposed the Cu:Mo concentration ratio in the diet as the best tool to estimate copper requirements in ruminants. A Cu:Mo ratio < 1 is indicative of high risk of copper deficiency, from 1 to 3 of marginal risk of copper deficiency, and a ratio > 3 is considered safe. Furthermore, the ratio Cu:Mo is also the most important dietary factor affecting copper toxicity in sheep, ratios of 10 or less will prevent toxicity in most cases (Berger and Cunha, 2006). Therefore, the practical outcome of molybdenum 'deficiency' in ruminants is a higher susceptibility to copper toxicity. Suttle (2010)—as well as other specialists in the field of trace elements in their textbooks, such as McDowell (2003), Anke (2004) and Kirchgessner (2011) – include molybdenum in the group of so-called 'occasionally beneficial trace elements'.

The content of molybdenum in soils and, consequently, in feed ingredients is highly variable depending on the geographical area (McDowell, 2003). The content in soils ranges 0.1–20.0 mg/kg dry matter (DM), with a median of 5 mg/kg DM (Suttle, 2010); molybdenum concentrations in feed ingredients are generally in the range of 0.2–2.5 mg/kg DM, and tend to be lower in grains and higher in legumes: detailed data are presented in Appendix A and Annex B. Standard molybdenum content in various feeds have been reported in Van Paemel et al. (2010) and extracted in Annex C. It is considered that if molybdenum concentrations in total feed are significantly lower than 1 mg, diets containing copper in the 10 mg/kg range may produce toxicity in sheep (NRC, 1985). Areas with low molybdenum concentrations (Cu:Mo ratios > 10) that can lead to excessive hepatic copper accumulation in sheep occur both outside (Australia, Canada) and within Europe (Great Britain, Scandinavia) (Gooneratne et al., 1989a). In the same way, besides low copper environmental concentrations, insufficient molybdenum intake in ruminants (that lead to copper toxicity) might result from pastures with a high content of clover (in which molybdenum is usually in the 0.1–0.2 mg/kg range; Todd, 1969) or from cereal-rich feeds, which are low in molybdenum and sulfur (Todd, 1972). In all these practical situations, sheep would benefit from a supplementation of molybdenum in the diet.

The additive under assessment is sodium molybdate dihydrate for use in feed for sheep. This inorganic molybdenum compound is already authorised in the EU as a nutritional feed additive for all animal species at the maximum level of 2.5 mg/kg in complete feed. The current assessment is on the re-evaluation of sodium molybdate dihydrate only for sheep.

3.1. Characterisation

3.1.1. Characterisation of sodium molybdate dihydrate

Sodium molybdate dihydrate (International Union of Pure and Applied Chemistry (IUPAC) name Sodium molybdate dihydrate; other names: Disodium molybdate dihydrate) is identified by Chemical Abstracts Service (CAS) number 10102–40–6 and the European Community number (EC-No.) 231–551–7. The chemical formula is $Na_2MoO_4{\bullet}2H_2O$. It has a molecular weight of 241.95 Da[10]; the theoretical molybdenum content is 39.65% (Figure 1).

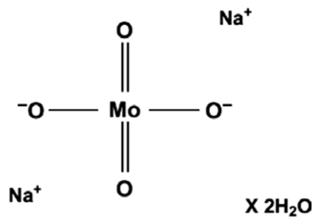

Figure 1: Molecular structure of sodium molybdate, dihydrate

Sodium molybdate dihydrate is a white crystalline powder. The applicant provided analytical data of five batches showing molybdenum content of 37.9–39.7% (specification ≥ 37%); the maximum content of water is 0.4%.[10] The pH is 9.0–10.0 in a 5% aqueous solution at 25°C; bulk density is 1,080 kg/m^3.[11]

Levels of heavy metals and arsenic were tested in three batches,[12] showing the following contents: lead 3–40 mg/kg, arsenic 10 mg/kg and cadmium 3–8 mg/kg. The levels of dioxins and the sum of dioxins and dioxin-like polychlorinated biphenyls (PCBs) measured in other three batches[13] were as follows: 0.09 ng World Health Organization-polychlorinated dibenzodioxin/dibenzofuran-toxic equivalents (WHO-PCDD/F-TEQ)/kg and 0.121–0170 ng WHO-PCDD/F-PCB-TEQ per kg, respectively. These levels are compliant with EU legislation.[14] Levels of mercury in the same three batches were 1 mg/kg, which appeared high compared with the levels permitted for feed materials (0.1 mg Hg/kg). The applicant provided analytical data on mercury in three additional batches of the additive[15] and the reported values were 0.06, 0.12 and 0.36 mg Hg/kg additive; the applicant highlighted the steps that were implemented in the manufacturing process, including those aimed to the removal of impurities such as heavy metals (see also Section 3.2.1). Considering also the proposed level of incorporation of the additive in complete feed (2.5 mg/kg complete feed), the resulting concentrations of mercury in feedingstuffs will be well below the limit allowed in the EU (0.1 mg/kg complete feed). The content of chromium and nickel was also analysed in the same three batches and the levels reported were below 2 mg/kg (limit of detection of the analytical method).[16]

[10] Technical dossier/Section II/Annex II_1.
[11] Technical Dossier/Section II.
[12] Technical Dossier/Section II/Annex II_2.
[13] Technical Dossier/Section II/Annex II_3.
[14] Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. OJ L 140, 30.5.2002, p. 10.
[15] Technical Dossier/Supplementary information/Annex B.
[16] Technical Dossier/Supplementary information/Annex C.

Particle size distribution was evaluated by laser diffraction on one batch showing that 0.22, 1.59 and 3.71% of the particles had a diameter below 10, 50 and 100 μm, respectively.[17] Data on particle size distribution on three additional batches was provided.[18] In one batch, the fractions with size up to 10, 50 and 100 μm diameter amounted to 1.64%, 8.74% and 22.35% (v/v), respectively. For the two other batches, the values were calculated for other particle size fractions: 5 and 20 μm diameter; total particle size distribution was given only by graphs, allowing an estimation of the fractions with size up to 10, 50 and 100 μm diameter to amount about < 2%, < 10% and < 20% (v/v), respectively.

The dusting potential was determined in the same additional three batches of sodium molybdate dihydrate and the values ranged from 1.86 to 3.03 g/m^3. The particle size distribution determined on dust of the same three batches ranged from 40.02% to 62.80% (v/v) for particles with diameter lower than 10 μm.[19]

3.1.2. Manufacturing process

Mined molybdenum ore is ground; by means of a physico-chemical process molybdenum disulfide (MoS_2) is separated and concentrated. The resulting molybdenum disulfide (MoS_2) is converted under high temperature to molybdenum trioxide (MoO_3). The MoO_3 solution is mixed with a sodium hydroxide (NaOH) solution to give the sodium molybdate. After the reaction has been completed, filtration takes place to remove possible physical impurities. Water is evaporated via a spin drying system and crystallization takes place. The product is sieved to remove coarse particles and homogenised.

The applicant provided a list of steps applied in the manufacturing process to obtain and purify MoO_3 from the mined molybdenum ore, aiming to reduce/remove impurities.[20]

3.1.3. Stability and homogeneity

Stability data are not required for inorganic compounds of trace elements. The maximum storage period recommended by the applicant is 12 months.[21]

To support the capacity of the additive to homogeneously mix, the applicant submitted data based on an experimental study carried out in a sheep premixture.[22] Ten premixture samples supplemented with sodium molybdate dihydrate (500 mg/kg corresponding to 200 mg Mo/kg premixture) were taken randomly and analysed for total molybdenum. The average level of molybdenum was 223.4 mg/kg with a coefficient of variation of 8.69%.

3.1.4. Physico-chemical incompatibilities

According to current knowledge, no incompatibilities or adverse interactions with feed components, carriers, other approved additives or medicinal products are to be expected for sodium molybdate in animal nutrition.

3.1.5. Conditions of use

The additive is intended to be incorporated via premixtures to complete feed for sheep up to a total maximum molybdenum concentration of 2.5 mg/kg complete feed. The use levels of the additive should consider the background levels of molybdenum, as well as the total copper level in feed (for details see Section 3.2.3 Safety for target animals and 3.3 Efficacy).

3.2. Safety

3.2.1. Absorption, distribution, metabolism and excretion (ADME)

Molybdenum in sodium molybdate dihydrate is present in the hexavalent form, being readily absorbed following oral administration. In humans, 30–70% of the dietary molybdenum is absorbed from the gastrointestinal tract (WHO, 1996), and specifically in the duodenum and proximal jejunum (European Commission, 2000). The absorption of orally administered water soluble forms of

[17] Technical Dossier/Section II/Annex II_6.
[18] Technical Dossier/Supplementary information/Annex D.
[19] Technical Dossier/Supplementary information/Annex E.
[20] Technical Dossier/Supplementary Information.
[21] Technical Dossier/Section II/Annex II_4.
[22] Technical Dossier/Supplementary Information/Annex F.

molybdenum (i.e. sodium molybdate, ammonium molybdate) is high, in the range of 75–97% for laboratory animals and ruminants (European Commission, 2000) and 88–93% for humans (Tallkvist and Oskarsson, 2015).

Intestinal absorption is affected by the presence of dietary copper and sulfur, especially sulfate. The complex pattern of interactions among these three elements has been reviewed in the EFSA opinion on the revision of copper levels in feed (EFSA FEEDAP Panel, 2016). Briefly, in the sulfide-rich environment of rumen, sulfur and molybdenum form thiomolybdates (mono-, di-, tri- and tetrathiomolybdates) which bind copper producing copper thiomolybdates that are not absorbed in the intestine. In the absence of rumen-available copper, thiomolybdates can be absorbed rapidly through the rumen wall or more slowly via the small intestine; then, thiomolybdates can bind to copper in biological molecules (Gould and Kendall, 2011).

Following gastrointestinal absorption, molybdenum appears rapidly in the blood predominantly bound as MoO_4^{-2} to α-2-macroglobulins, but partly also to the erythrocyte membrane (Barceloux, 1999). At low molybdate and sulfur uptake more than 70% of molybdenum is attached to the membrane of the erythrocytes. On the other hand, when the supply of both elements is high, the molybdenum is predominantly bound to α-2-macroglobulins in the plasma (Underwood, 1977).

During the 2007–2010 period, Wiese (2014) determined molybdenum in plasma (1–32 μg/L), serum (1–31 μg/L) and urine (23–323 μg/L) of cows from 489 dairy farms in East Germany; the author found a good agreement with literature published data as well as with recommended reference range values. Molybdenum concentrations in liver biopsies obtained from 40 animals showed a mean value of 3,447 μg/kg DM and ranged from 661 to 6,239 μg/kg DM).

Distribution studies in laboratory and farm animals showed that molybdenum rapidly appears in kidneys, liver and bone (Tallkvist and Oskarsson, 2015). In humans, the highest levels also appear in kidney, liver and bone; high levels appear also in adrenals, fat and omentum. Although molybdenum crosses placenta (European Commission, 2000) it seems that it occurs at a small extension (Vyskocil and Viau, 1999). Molybdenum retention occurs partly through formation of the molybdopterin complex (IOM FNB, 2001).

Molybdenum may influence the metabolism of other electrolytes in ruminants. High molybdenum intake may reduce the phosphorus concentration in plasma (Underwood, 1977; cited in Wiese, 2014); under this condition, there is an increased excretion of phosphates via faeces in cattle (Pitt, 1976; cited in Wiese, 2014).

Molybdenum intake and excretion are balanced in most non-ruminant species, including humans (WHO, 1996). The major part of the administered metal is excreted within a few hours (Rajagopalan, 1988) and there is no apparent bioaccumulation of molybdenum and the tissue levels rapidly return to normal once exposure stops (European Commission, 2000). On the contrary, increased retention of molybdenum is described in ruminants with increasing sulfur intake. When sulfate and molybdate are added simultaneously to the diet of sheep, molybdenum retention increases; in the meanwhile, faecal excretion increases and urine excretion decreases (Suttle, 2010). This increased retention is likely due to the absorbed thiomolybdates that readily bind to copper-containing biological molecules.

Molybdenum is primarily excreted through urine (Tallkvist and Oskarsson, 2015). The mechanism of excretion by the kidney is likely the same in ruminants and non-ruminants. The interaction of molybdenum with sulfur can occur also in the kidney and influence excretion, due to the competitive inhibition of molybdate reabsorption by sulfate, as shown in sheep (Bishara and Bray, 1978). The molybdenum excreted via the faeces is partly unabsorbed molybdenum and partly secreted through the bile (Vyskocil and Viau, 1999). In humans, 17–80% of the total dose is excreted via urine while 1% or less via the bile (Vyskocil and Viau, 1999).

In ruminants, the molybdenum content of milk is low (Friesecke, 1994); appreciable molybdenum excretion in milk can only be observed following a high molybdenum intake (Hogan and Hutchinson, 1965; Palmer et al., 1989).

3.2.2. Toxicological studies

3.2.2.1. Reviews on molybdenum toxicology

The main adverse effects of molybdenum observed in laboratory animals at repeated low dose exposure (2–8 mg Mo/kg bw per day in rats and rabbits) are growth decrease and alterations in development. Developmental changes were observed at 1.6 mg kg/day in rats. At higher doses (8–50 mg Mo/kg bw per day in rats; 23 mg Mo/kg bw per day in rabbits) male infertility, testicular degeneration, anorexia and weight loss were reported (Vyskocil and Viau, 1999). In this review, the authors considered as critical the toxic effects of molybdenum for reproduction and fetal development in rats observed by Fungwe et al. (1990). Briefly, female rats were exposed to molybdenum in drinking water during 9 weeks at concentrations corresponding to the calculated dose levels of 0.9, 1.6, 8.3 and 16.7 mg Mo/kg bw per day. The animals were mated with unexposed males and at 21 day of gestation were anaesthetized and submitted to laparotomy. At 1.6 mg Mo/kg bw per day and higher dose levels, oestrus cycle was prolonged, gestational weight, litter size and fetal weights were lower than controls and fetal resorption increased. Histopathology showed delayed histological development of fetal structures, delayed oesophageal development, delayed transfer of fetal haematopoiesis from liver to bone marrow and delayed myelination of the spinal cord at doses ≥ 1.6 mg/kg bw per day; the no observed adverse effect level (NOAEL) was set at 0.9 mg Mo/kg bw per day. Based on the NOAEL of 0.9 mg Mo/kg bw per day for reproductive and developmental toxicity and the conventional safety factor of 100, Vyskocil and Viau (1999) proposed a human Tolerable Daily Intake (TDI) of 0.009 mg Mo/kg bw per day.

In 2000, the Scientific Committee on Food (SCF) set an upper intake tolerable level (UL) for adults of 0.01 mg/kg bw per day for molybdenum (0.6 mg/day); this value was derived from the same reproduction toxicity study in rats described above where a NOAEL of 0.9 mg/kg bw was identified (Fungwe et al., 1990). For children the UL was set between 0.1 and 0.5 mg/day. The conclusions by Vyskocil and Viau (1999) and the SCF (European Commission, 2000) were also endorsed in a more recent review on the chemistry and biology of molybdenum (Tallkvist and Oskarsson, 2015). The FEEDAP Panel notes that the available evidence does not allow to conclude on a possible role of Mo-induced copper deficiency in the reproductive and developmental toxicity of molybdenum: however, copper deficiency is recognized as a risk factor for pregnancy and fetal health in mammals, including humans (Gambling et al., 2011).

In the opinion of the ANS Panel on potassium molybdate as a source of molybdenum added for nutritional purposes to food supplements (EFSA, 2009), the Panel generally shared the SCF opinion of 2000 and included a set of genotoxicity studies (*in vitro* and *in vivo* micronucleus, *in vivo* dominant lethal assay), that was not examined in previous assessments. The Panel concluded that the genotoxicity data needed further investigation, since the *in vitro* assay was positive and the two *in vivo* assays were borderline positive (Titenko-Holland et al., 1998). Even taking into account the high dietary intake range for the European population of 96–500 μg Mo/day, the EFSA ANS Panel concluded that the intake by adults of 20 μg Mo/day as a food supplement did not exceed the UL of 0.6 mg/day established by SCF in 2000.

In 2013, the EFSA NDA Panel delivered an opinion on the dietary reference values (DRVs) for molybdenum (EFSA NDA Panel, 2013). In the absence of human data to derive a molybdenum requirement, an AI of 65 μg/day was proposed for adults and suggested that this value also applies to pregnant and lactating women. An AI was also proposed for infants from seven months and for children based on extrapolation from the adult AI using isometric scaling and the reference body weights of the respective age groups.

Other scientific bodies, as the Food and Nutrition board of the Institute of Medicine, reported a molybdenum daily intake from food in the US of 109 and 76 μg for men and women, respectively, and established a recommended dietary allowance of 45 μg/day for adults (IOM FNB, 2001). The IOM derived a UL of molybdenum of 2,000 μg per day. This UL was based on the NOAEL identified by Fungwe et al. (1990) (0.9 mg/kg bw and day, described above); a composite uncertainty factor of 30 (10 for interspecies differences and 3 for intraspecies variability) was applied and the value was corrected to a human adult body weight of 68.5 kg.

The Expert group on vitamins and minerals (EVM, 2003) from the UK concluded that there are insufficient data to establish a safe upper level for molybdenum; the maximum estimated dietary intake of 230 μg/day was considered not to pose a health risk.

Based on toxicological human data of molybdenum and the reference dose calculated by the US Environmental Protection Agency, the Council of Responsible Nutrition proposed 350 μg Mo/day for supplemental intake (Hathcock, 2013).

3.2.2.2. Genotoxicity

The genotoxicity of molybdenum compounds has been addressed by the SCF (European Commission, 2000), by the EFSA ANS Panel (EFSA, 2009) and by the WHO (2011), all reporting conflicting, both positive and negative, results. WHO and SCF stated that 'Ammonium molybdate was mutagenic in two of three *Escherichia coli* strains. Molybdenum(V) chloride was negative and ammonium molybdate strongly positive in the *Bacillus subtilis* rec-assay using deoxyribonucleic acid (DNA) repair-competent H17 and repair-deficient M45 strains (Nishioka, 1975). Ammonium and sodium molybdates were neither mutagenic nor recombinogenic in the *Saccharomyces cerevisiae* reverse mutation and gene conversion assays (Singh, 1983)'.

The EFSA ANS Panel (EFSA, 2009) made reference to a publication of Titenko-Holland et al. (1998) with sodium molybdate. The authors reported the results of an *in vitro* micronucleus assay in human lymphocytes and two *in vivo* tests, a micronucleus assay in mouse bone marrow and a dominant lethal assay in mouse. A modest but significant concentration dependent induction of micronucleated cells was observed *in vitro*. For both *in vivo* assays, mice were given intraperitoneally 200 or 400 mg sodium molybdate/kg bw. In the micronucleus *in vivo* assay, also a weak genotoxicity effect was detected (twofold increase of micronucleated cells as compared with 3.5-fold increase for the positive control). In the dominant lethal assay, an overall dose-dependent increase was observed in total post-implantation loss. The FEEDAP Panel notes that these two positive *in vivo* studies were carried out by intraperitoneal administration, not reflecting for consumer exposure. However, the EFSA ANS Panel noted that these genotoxicity data might need further investigation.

A recent publication (Burzlaff et al., 2017) reported negative results in three *in vitro* assays performed with sodium molybdate, both in the presence and in the absence of metabolic activation: bacterial reverse mutation assay (in *Salmonella* Typhimurium strains TA98, TA100, TA1535, TA1537 and TA102), mammalian cell gene mutation assay in mouse lymphoma L5178Y cells and *in vitro* micronucleus assay in cultured human peripheral blood lymphocytes.

The available data set on the genotoxicity of molybdenum compounds remains conflicting. The FEEDAP Panel deems that the genotoxic activity reported both *in vitro* and *in vivo* has no obvious alternative explanation and should not be disregarded.

Molybdenum is a transition metal and as such is a potential inducer of possibly genotoxic reactive oxygen species. Also other molecular mechanisms have been proposed to explain the genotoxicity observed in the experimental studies, including the unbalance of the pool of DNA precursors caused by the increase of xanthine oxidase activity and the interaction with proteins involved in mitosis leading to aneuploidy (Titenko-Holland et al., 1998). All these suggested mechanisms envisage a threshold, therefore the mutagenic activity would be expressed only at relatively high levels of exposure; such levels are unlikely to be reached in the conditions of consumer exposure to molybdenum from its use as a feed additive.

3.2.2.3. Repeated dose toxicity studies in rats

A 90-day toxicity study was performed in rats after oral administration of sodium molybdate in diet corresponding to 0, 5, 17 or 60 mg Mo/kg bw per day (Murray et al., 2014). For the higher dose group, some reproductive parameters were also determined. The authors found a NOAEL of 17 mg Mo/kg bw per day based on effects on body weight, body weight gain, food conversion efficiency and renal histopathology (females only) at 60 mg Mo/kg bw per day. Based on the absence of adverse effects on reproductive organ weights and histopathology, oestrus cycles and sperm parameters at all tested doses, the study produced a NOAEL of 60 mg Mo/kg bw and day for reproductive toxicity, much higher than the previous one of 0.9 mg/kg bw found by Fungwe et al. (1990) and adopted by SCF 2000 to set the UL of 0.01 mg/kg bw per day for molybdenum (0.6 mg/day). However, the effects of molybdenum on pregnancy and embryogenesis were not evaluated in this study. The FEEDAP Panel considers that this study does not provide a full picture of reproductive and developmental effects of molybdenum; thus, the NOAEL derived from this study cannot supersede the NOAEL used by the SCF.

A study was conducted to evaluate the effects of molybdenum on male fertility (Pandey and Singh, 2002). Male rats were orally given, during 60 days, 5 days per week, sodium molybdate at doses of 10, 30 and 50 mg/kg bw per day (corresponding to approximately 4.7, 14 and 23.3 Mo/kg bw per day). The animals were killed on day 61 and testes, epididymis, seminal vesicles and prostate glands were removed. Relative and absolute organ weights of epididymis, seminal vesicle and prostate were significantly reduced at 30 and 50 mg/kg. Testis relative weight was also significantly reduced at 50 mg/kg. Both sperm motility and total sperm count were significantly reduced at the doses of 30 and 50 mg/kg. Significant morphological abnormalities in spermatozoa were observed at 30 and 50 mg/kg. The activities of the testicular enzymes sorbitol dehydrogenase, lactate dehydrogenase and γ-glutamyltranspeptidase were significantly altered on a dose-dependent manner. The two higher doses caused histological alterations of seminiferous tubules and testes. In rats given the 50 mg/kg molybdate, molybdenum significantly accumulated in epididymis, seminal vesicles and prostate gland. Males dosed with 30 mg Mo/kg bw were mated with unexposed females and on day 20 of gestation laparotomies were performed for parameters evaluation. At the level of 30 mg Mo/kg, fertility index was 60% as compared to 80% in control animals. Implantation, pre-implantation loss, live fetuses, number of resorptions, post-implantation loss, fetal crown-rump length and fetal weight were significantly reduced. Overall, this study evidenced that molybdenum affects male fertility in a dose-dependent manner and that also the progeny may suffer from male mediated effects. The lower dose level did not elicit any adverse effects on the evaluated parameters; therefore the NOAEL for male fertility was 10 mg sodium molybdate/kg bw per day, corresponding to 4.7 mg Mo/kg bw per day.

In conclusion, taking into account the previous safety assessments of molybdenum, the pivotal study of Fungwe et al. (1990) on fertility in females (NOAEL of 0.9 mg/kg bw per day) which was the basis for the UL calculations, and the fertility study on males by Pandey and Singh (2002) (NOAEL of 4.7 mg Mo/kg bw per day), the FEEDAP Panel agrees with previous evaluations that identified the adverse effects on reproduction as the critical ones to set the NOAEL, selecting the lowest value of 0.9 mg Mo/kg bw per day.

3.2.2.4. Chronic toxicity and carcinogenicity studies

To the knowledge of FEEDAP Panel, there are no chronic studies with molybdenum administered by oral route to laboratory animals.

The NTP (1997a,b) carried out a 2-year study in rats and mice exposing the animals by inhalation to molybdenum trioxide. The respective data is described under 'Safety for User' (see Section 3.2.5).

3.2.2.5. Information on oral toxicity in humans

Limited data indicate a low toxicity of molybdenum compounds in humans where exposure is by ingestion (Vyskocil and Viau, 1999). Food or water containing levels higher than 100 mg Mo/kg can cause signs of toxicity, including diarrhoea, anaemia and high levels of uric acid in blood (EVM, 2003).

In 2011, the WHO set a safe limit for molybdenum in drinking water of 0.07 mg/L based on a 2-year epidemiological human study carried out in the USA (WHO, 2011). Two groups of young people were exposed to molybdenum in water (1–50 ug/L and 200 ug/L or higher). Higher mean serum ceruloplasmin (401 vs 30 mg/100 mL) and lower mean serum uric acid (4.4 vs 5.3 mg/100 mL) were associated with the higher molybdenum intake. Because no adverse effects were seen in either group, a NOAEL for molybdenum in drinking water of 200 μg/L was derived. An uncertainty factor of 3 was applied to reflect intraspecies variation; based on the very limited information provided the study did not investigate endpoints relevant to reproductive health. This value would correspond to approximately 0.002 mg/kg bw in a 60-kg individual. Since this value concerns drinking water only, it is compatible with the toxicologically-based overall safe intake of 0.01 mg/kg bw as set by the SCF. The FEEDAP Panel notes that no human epidemiological studies investigated the potential effects of molybdenum exposure on reproduction and development.

3.2.3. Safety for the target animals

No tolerance study conducted with the additive under assessment was provided to support the safety of the additive for sheep. The FEEDAP Panel notes that in the case of molybdenum compounds, safety for target species and efficacy are highly interrelated and thus accepted the approach of the applicant to provide a literature search to support simultaneously the safety and the efficacy of molybdenum compounds (including sodium molybdate) for sheep.[20] The search was done in three databases: Web of Science, Toxline and Google scholar considering and including until May–August 2016; the keywords and the string and Boolean operators used were described. A total of 2,260 references were retrieved; from those, the applicant considered relevant ten references and focused nearly exclusively on the risk of copper poisoning in ruminants (related to low molybdenum content or imbalanced Mo:Cu ratios in the diet) and the use of sodium molybdate (in the diet or as medical treatment) to prevent or treat copper excess in animals. The information properly related to safety for target species provided by the applicant was very limited, as e.g. no details of other dietary constituents were given. Also findings were contradictory, whereas according to Ward (1978) molybdenum concentration required to produce copper deficiency is in the range of 100–200 mg/kg DM, for Gooneratne et al. (1989a) 5 mg/kg diet are sufficient to affect copper homeostasis.

The most comprehensive review of maximum tolerable levels (MTL, expressed as concentrations in complete feed) of molybdenum in animals has been carried out by the National Research Council (NRC) in its revision of 2005 (NRC, 2005). It is generally assumed that, except for ruminants, molybdenum at concentrations usually found in feeds has a relatively low toxicity for most animal species. On the contrary, the complex non-competitive interaction among molybdenum, copper and sulfur in ruminants makes this animal species very sensitive to molybdenum toxicity under some practical conditions (Spears, 2003). Even though information available for sheep is more limited than for cattle, the mechanism of toxicity is the same for both species (NRC, 2005; Berger and Cunha, 2006) and related to a secondary copper deficiency (molybdenosis). A detailed appraisal of molybdenum toxicity in ruminants is provided in Appendix B. Briefly, the ruminal microbial metabolism favours the reaction of sulfur and molybdenum enabling the formation of thiomolybdates which bind copper with high affinity and prevent copper intestinal absorption. In the absence of rumen-available copper, thiomolybdates can be rapidly absorbed and bind copper in biological compounds, resulting in an increase of copper biliary excretion from liver stores, a reduced transport of copper for biological processes and a remove of copper from cupro-enzymes. Mild molybdenosis may be identified only by biomarkers such as increases in xanthine oxidase activity or blood uric acid. On the contrary, clinically evident molybdenosis may be severe, with significant mortality unless intervention with supplemental copper and/or removal from pasture was performed. Three scenarios for the occurrence of molybdenosis can be identified: (i) high molybdenum intake alone, (ii) moderately high molybdenum intakes concomitant with low dietary copper levels, or (iii) imbalanced dietary Cu:Mo ratios. High molybdenum intake alone (100 mg/kg diet DM and above) cause molybdenosis regardless of the copper and sulfur status. Clinical toxicosis may also be produced in cattle and sheep by 25–50 mg Mo/kg DM. However, when the dietary copper level falls below normal and/or the sulfate level is high, molybdenum intakes between 5 and 20 mg/kg diet, and even as low as 1 or 2 mg/kg, may exert adverse effects. Based on the available information, the NRC established a MTL of molybdenum at 5–10 mg/kg of DM for cattle with an adequate dietary copper intake and, in spite of the limited data, considered safe to suggest the same level for sheep. However, unless dietary copper is increased above requirement, this concentration of molybdenum may lead to copper deficiency over time.

In addition to the Mo–Cu–S relative concentrations in the diet, pasture type and preservation techniques determine different degrees of Mo–Cu–S antagonism. The effects of molybdenum and sulfur on copper absorption (as determined by plasma copper repletion rates in hypocupraemic Scottish Blackface sheep) in grasses, hays and silages are described in Suttle (2010) with prediction equations. Overall, the effect of sulfur and molybdenum varies depending on the feedstuff serving as a source of copper: (i) in silages, molybdenum has a small effect, but sulfur reduces copper absorption in a logarithmic manner; (ii) in hays, the inhibitory effect of molybdenum and sulfur is relatively low; and (iii) in fresh grass copper absorption is greatly reduced by small increments in molybdenum and sulfur contents and, similarly, both sulfur and molybdenum markedly affect copper absorption also in concentrate-type diets (Suttle, 2010).

Moreover, the large differences among sheep breeds in regard to the susceptibility to copper unbalances represent a further factor of uncertainty in determining with precision a MTL for molybdenum in sheep feed. Based on the capacity to accumulate copper in liver, ovine breeds have been classified as tolerant or resistant to copper deficiency/toxicity; for instance, the Texel breed has a high tendency to accumulate copper whereas the Scottish Blackface breed is highly susceptible to copper deficiency (summarised in EFSA FEEDAP Panel, 2016).

For practical use, the Cu:Mo ratio (in mg/kg DM) in the feed is used to predict risk for copper deficiency and toxicity. Although these ratios require flexible interpretations, ratios < 1 generally indicate a high risk of copper deficiency, whereas ratios of 1–3 indicate a marginal risk of copper deficiency (Suttle, 2010). On the other hand, the ratio Cu:Mo is also the most important dietary factor affecting copper toxicity in sheep, ratios of 10 or less will prevent toxicity in most cases; the optimal Cu:Mo ratio in sheep is in the range of 3–10 (Berger and Cunha, 2006), which can take into account also interbreed differences in copper metabolism and retention.

Some few studies suggest that molybdenum may be a reproductive toxicant in ruminants (study in sheep: Mills and Fell, 1960; study in calves: Thomas and Moss, 1951; both cited in European Commission, 2000). The FEEDAP Panel retains that, besides the fact that the studies are rather old, the levels of molybdenum tested are much higher than those currently authorised in the EU (e.g. in the range of 5–100 mg Mo/kg dry matter; see Appendix B for more details). Molybdenum toxicity in farm animals is manifested as antagonism of absorption and/or biological activity of copper, and is greatly enhanced by high sulfur content in the diet. Ruminants, including sheep, are highly susceptible to molybdenum excess, which may induce a clinically severe copper deficiency (molybdenosis). Whereas molybdenosis can be induced by high intakes of molybdenum alone (100 mg/kg diet DM and above), levels below 5 mg/kg diet and as low as 1 or 2 mg/kg may exert adverse effects in ruminants, including sheep, when the dietary copper level falls below normal and/or the sulfate level is high. Conversely, low molybdenum in the diet is expected to enhance copper toxicity, if the intake of copper is high. The FEEDAP Panel also notes that whereas molybdenum is an established reproductive toxicant in laboratory rodents (see Section 3.2.2.3), not enough evidence exists to identify possible reproductive hazards, if any, in ruminants bred for reproduction.

3.2.3.1. Conclusions on the safety for sheep

The FEEDAP Panel considers that it is difficult to establish a 'generally safe' dose of molybdenum in sheep. This is due to the molybdenum dependence on the copper and sulfur intake. The optimal Cu:Mo ratio in sheep is in the range of 3–10; a Cu:Mo ratio below 3 and especially below 1 is associated with an increased risk of Mo-induced copper deficiency, whereas a ratio over 10 indicates an increased risk of copper toxicity.

The highest total copper level authorised in complete feeds for sheep is 15 mg/kg. Considering the potential range of content of copper in practice in complete feed for sheep, the highly variable susceptibility to copper toxicity of sheep breeds and the biological interactions of sulfur with copper and molybdenum, the FEEDAP Panel considers that the current maximum authorised molybdenum content of 2.5 mg/kg feed is adequate and safe for the range of copper content in feeds between 8 (corresponding to basal requirement) and 15 (maximum authorised content).

3.2.4. Safety for the consumer

3.2.4.1. Deposition studies

Background molybdenum levels are normally present in edible animal tissues. Limited studies indicate that molybdenum content in tissues can be influenced by levels in feed; however, accumulation occurs to a low extent only and levels return to background upon the withdrawal of supplementation. Souci et al. (2008) reported some data on the normal presence of molybdenum in edible animal tissues from pigs and cattle: muscle 30 μg/kg (pigs), kidney 540 and 590 μg/kg (cattle and pigs, respectively), liver 1,650 and 2,000 μg/kg (cattle and pigs, respectively). These figures in liver are consistent with other data (Berger and Cunha, 2006) giving molybdenum levels present in liver of animals (species not mentioned) of 2,000–4,000 μg/kg. Wiese (2014) found significant correlations (coefficient R) ($p < 0.01$) between molybdenum concentration in the liver of cows (reference values of molybdenum in the cows' liver as 1,000–4,000 μg/kg DM) and some other mineral elements: 0.62 for Mo:Cu; 0.56 for Mo:Mn; 0.69 for Mo:Mg and 0.60 for Mo:K.

According to Souci et al. (2008), the normal values for goat's and cow's milk are 18 and 42 μg/L, respectively. Underwood (2012) reported a range of molybdenum in goat's milk of 11–16 μg/L and for cow's milk 40–56 μg/L; in another study, 24–60 μg Mo/kg milk was described (Zamberlin et al., 2012). Other studies provide evidence that molybdenum in milk rapidly raises when dietary molybdenum is high. In sheep milk, Hogan and Hutchinson (1965) found values of < 10 μg/L (unsupplemented-Mo feed) to 980 μg/L with 12.5 mg Mo/kg complete fed. Palmer et al. (1989) reported a range of molybdenum concentration in normal cow's milk between 18 and 120 μg/kg; the highest level of molybdenum (2,100 μg/kg) was found for a cow fed 200 mg Mo/kg feed. The authors concluded that the transfer rate of dietary molybdenum to milk under these conditions may amount to about 1%. This is in line with a study published in the 1970s reporting a transfer of molybdenum in dairy goat's milk of approximately 2% (Anke et al., 1971).

Pott et al. (1999) provided data on the effect of various molybdenum sources and levels in complete feed on tissue molybdenum concentrations in lambs (Table 1).

Table 1: Concentration of molybdenum in tissues and serum of lambs, following the feeding with various sources and levels of molybdenum in feed (Pott et al., 1999)

Mo source	Molybdenum in complete feed[(a)] (mg Mo/kg)	Molybdenum in tissues (mg/kg DM)			Molybdenum in serum (mg/L)
		Liver	Kidney	Muscle	
Control	1.2	3.34	1.64	0.11	0.007
Sodium molybdate	1.2 + 15	6.0	2.8	0.28	0.28
	1.2 + 30	7.52	4.3	0.44	0.48
	1.2 + 45	7.77	10.64	0.67	1.2
Ammonium molybdate	1.2 + 30	8.38	6.05	0.49	0.85
Molybdate trioxide	1.2 + 30	9.24	6.9	0.56	0.81
Molybdenum metal	1.2 + 30	5.51	1.7	0.27	0.01
Pooled SE		0.21	0.34	0.02	0.03

DM: dry matter.
(a): Includes molybdenum background and supplementation.

Data in Table 1 show that a supplementation of 15 mg Mo (from sodium molybdate)/kg feed, which is sixfold higher than the current maximum authorised level of 2.5 mg/kg, causes an approximate twofold increase of molybdenum levels in liver, kidney and muscle, compared to the background (1.2 mg/kg feed). Greater supplementation (30 and 45 mg/kg feed) led to a dose-related increase of molybdenum concentrations in kidney and meat, whereas the increase was less appreciable in liver. In both control and 15 mg/kg supplemented lambs' feed, liver and kidney contained molybdenum concentrations approximately 20- and 10-fold higher, respectively, than muscle.

These data suggest that the maximum authorised level of 2.5 mg of Mo/kg feed would have only a limited impact, if any, on the concentrations of molybdenum in edible sheep tissues.

3.2.4.2. Assessment of consumer safety

The EFSA NDA Panel was requested to derive DRVs for molybdenum (EFSA NDA Panel, 2013). The NDA Panel stated that the evidence required to derive an Average Requirement and a Population Reference Intake was considered insufficient; therefore, the NDA Panel proposed an AI for adults (including pregnant and lactating women) of 65 μg/day and 15 μg/day for toddlers.

The NDA Panel reviewed ten nutrition surveys for molybdenum intake of adults. The results were fairly consistent across countries and sampling years: the range (μg/day) was between 74 ± 62 (Germany, sampling of 1988) and 112 ± 63 (Denmark, sampling of 1988). Additionally, in the United Kingdom (sampling of 2006), an intake of 1.61–1.64 μg/kg body weight per day (110–115 μg/day in a 70-kg person) was reported. A Total Diet Study performed in UK on 1994 gave a 97.5th percentile intake of 210 μg/day (average 111 μg/day) (EVM, 2003). Foods high in molybdenum are pulses, cereal grains and grain products, offal (liver, kidney) and nuts. Cereals and cereal-based products including bread are the major food contributors to dietary molybdenum intake of adults and contribute about one-third to one half of total molybdenum intake. In Armenia, a country neighbouring EU, a recent paper estimated that the average Mo intake from vegetables (including legumes, leafy and root vegetables) is in the range of 20 μg/day in adults of both sexes; cabbage and beans (approx. 8 and 5 μg/day, respectively) were main contributors (Pipoyan et al., 2018).

The NDA Panel gave details on the contributions from the several major foods of animal origin as derived from surveys in UK (published 2010), France (published 2011) and Sweden (published 2012). The contribution percentages were 12% in France (milk 4, dairy products other than cheese 4, cheese 2, delicatessen meat 2), 17% in UK (milk 7, dairy products 4, meat products 4, poultry 1, eggs 1) and 22% for Sweden (dairy products 16, meat 5, eggs 1). It may be noteworthy that, whereas excretion of molybdenum in milk is low, milk and dairy products altogether may make up to 15% of the total molybdenum intake. Also, whereas liver and kidney are considered as relatively rich molybdenum sources their contribution to the total molybdenum intake in the general population is presumably limited.

Data on molybdenum content of edible tissues have been summarized above in Section 3.2.4.1 (Deposition Studies). In particular data on molybdenum in sheep liver have been provided by Berger and Cunha (2006); even though no data for mutton and sheep milk were available, it can be reasonably assumed, based on available evidence, that molybdenum levels are comparable to those observed in cattle (Souci et al., 2008).

Consumption of sheep products likely show significant national and regional differences across the EU, reflecting the variability in sheep production (EFSA AHAW Panel, 2014); while in several countries sheep products are minor food commodities, in other countries sheep-derived food may represent a significant component of the overall meat and dairy product consumption. This assumption is supported by the data available in the EFSA comprehensive database, although these figures do not cover all EU countries.

Table 2 reports the highest figures of consumption for meat and edible offal from sheep observed in Countries from where data have been made available and where the reported percentage of consumers of sheep meat was at least 5%.

Table 2: Data from consumers and consumption of meat and edible offal of sheep in EU Countries extracted from the EFSA comprehensive database

Country	Age group	Consumers (%)	Consumption (g/day)	
			Median	P95
Belgium	Adults	6.4	55.5	131.5
Denmark	Adults	8.6	22.2	57.8
Spain	Adults	10.4	64.2	115.5
Finland	Toddlers	5.2	9.5	19.1
France	Adults	27.5	12.2	43.1
UK	Adults	25.2	18.2	47.6
Greece (Crete)	Children	17.8	25.0	66.7
Ireland	Very elderly	27.3	50.7	102.8
Latvia	Pregnant women	6.3	34.5	55.2
Sweden	Elderly	6.1	29.4	94.5

The consumption of sheep liver and kidney is usually restricted to a fraction of consumers below 1%. The highest available figures for liver consumption are from UK (adults: 2.3% of consumers, median 17.9 g/day, P95 42.9; very elderly: 5% of consumers, median 21.8 with P95 of 64) and Ireland (adults: 3.3% of consumers, median 17.4 g/day, with P95 of 62.7). The highest available figures for kidney are from Ireland (elderly: 4.7% of consumers, median 19.0 g/day, with P95 of 50) and UK (adults: 3.5% of consumers, median 3.46 g/day, with P95 of 11.4).

Assuming contents of molybdenum in tissues of 30 μg/kg in muscle, up to 600 μg/kg in kidney and up to 4,000 μg/kg in liver (see above Section 3.2.4.1), the highest consumption figures would be 3.3 μg/day for meat (P95 Spanish adults), 240 μg/day for liver and 30 μg/day for kidney (both for P95 Irish elderly). Considering the high consumers of sheep offals within the elderly Irish population as a subgroup with very high molybdenum intake, the resulting 270 μg/day would be less than 50% of the 0.6 mg/day (or 600 μg/day) UL. Since the intake of molybdenum from other dietary sources is very unlikely to exceed 150 μg/day, the FEEDAP Panel considers that the consumption of sheep liver and kidney would not lead to a molybdenum intake higher than the UL even in high consumers.

The FEEDAP Panel notes that data on the consumption of sheep dairy products are very limited, with the exception of data on consumption on yoghurt from sheep milk in a few countries: the most highest figure is from Cyprus (adolescents, 31.4% consumers) with a P50 and P95 of 15.0 and 96.7 g, respectively; Cyprus was also the only surveyed Country where the fraction of consumers of sheep-derived yoghurt was above 3%. Data on consumption of liquid milk from Germany and Spain indicated that consumers were below 0.5% of the population. The FEEDAP Panel notes that, indeed, the usual pattern of consumption of sheep milk in the EU Countries is usually as cheese. The daily consumption of 100 g of a sheep dairy product (P95 of Cyprus consumption figure) containing an extreme concentration of 60 μg/day (Zamberlin et al., 2012) would lead to an intake of 6 μg/day, representing 1% of the UL. The FEEDAP Panel notes that the available data may lead to an underestimation of the molybdenum intake through dairy sheep products in some EU countries, in particular due to lack of sheep cheese consumption figures; while this underestimation is difficult to evaluate, such uncertainty is unlikely to have any influence on the conclusion that the consumption of sheep-derived foods does not lead to a molybdenum intake higher than the acceptable daily intake (ADI).

Toxicokinetics data in laboratory rodents and farm animals (including sheep), however incomplete, uniformly indicate that molybdenum would not accumulate in edible tissues or products of animal fed molybdenum supplemented diets up to the maximum level in feed of 2.5 mg/kg.

The FEEDAP Panel retains the UL of 0.01 mg/kg bw for molybdenum established by the SCF, equivalent to 0.6 mg/60-kg person per day for adults (European Commission, 2000; see Section 3.2.2.1). The FEEDAP Panel regards the UL set by the SCF as provisional; the Panel considers that this UL should be re-examined when further data become available to assess whether molybdenum has a genotoxic potential by oral route as well as, if this is the case, the mode of action (e.g. direct clastogenic effect or chromosome damage secondary to Mo-induced copper deficiency) of genotoxic effects.

Based on the limited data available (EFSA NDA Panel, 2013), consumer exposure in the EU is not associated with a risk of excess molybdenum intake in the general population. Since sodium molybdate has an established, long-term use in food-producing animals in EU, the estimates of dietary molybdenum intakes in European consumers have incorporated foods from animals fed molybdenum-based feed additives, including any impact of the use of sodium molybdate on the molybdenum content of edible tissues and products. Although no data on the molybdenum content of sheep-derived food in relation to feeding conditions in accordance with current EU regulation are available, the supplementation levels in sheep are very unlikely to pose a risk to consumers, considering also the low contribution of sheep derived food to the total molybdenum exposure of consumers.

Therefore, the FEEDAP Panel considers that the use of sodium molybdate as a feed additive for sheep at the maximum level in feed of 2.5 mg/kg is safe for consumers.

3.2.4.3. Conclusions on safety for the consumer

As other essential trace elements, molybdenum is required at very low levels; higher concentrations have no nutritional value and, above a certain level, are potentially toxic.

Toxicokinetic data in laboratory rodents and farm animals (including sheep), however incomplete, uniformly indicate that molybdenum would not accumulate in edible tissues or products of sheep fed molybdenum supplemented diets up to the upper maximum level of 2.5 mg/kg. Based on available data on the molybdenum content in foods of animal origin and on the consumption of sheep-derived foods in different EU countries, and assuming that the estimates of dietary molybdenum intakes have incorporated foods from animals fed molybdenum-based feed additives, the consumption of sheep-derived foods would not lead to an intake above the UL (0.01 mg/kg bw) even in high consumers.

The FEEDAP Panel considers that the use of sodium molybdate as a feed additive in sheep at the currently maximum authorised level in feed of 2.5 mg/kg is safe for consumers.

3.2.5. Safety for the user

3.2.5.1. Inhalation toxicity

No inhalation toxicity study with sodium molybdate dihydrate was presented.

There is long history of exposure to molybdenum in industrial workers. No distinct occupational syndrome due to handling of molybdenum compounds is known. Since potential effects on the respiratory system are likely related to molybdenum itself, information on other molybdenum compounds can be read-across to evaluate the inhalation hazard by sodium molybdate.

The National Toxicology Program (NTP) carried out a thorough set of inhalation toxicity studies on molybdenum trioxide (99% purity) on rats and mice for 14 days, 13 weeks or 2 years (NTP, 1997a,b).[23] The results indicate that in rodents adverse effects in the respiratory tract, including carcinomas, are induced by life-long (2 year) exposures to 10 mg/m^3 molybdenum trioxide and higher. No such effects, nor other significant adverse effects, were observed upon a short-term (14 days) or a subchronic (13 weeks) exposure to up to 100 mg/m^3. Therefore, based on the NTP results, molybdenum trioxide elicits inhalation toxicity in rodents only upon chronic lifelong exposure, likely linked to continuous and protracted site-of-contact toxicity in the respiratory tract mucosae.

In humans, acute inhalation symptoms are cough and a sore throat (IPCS, 2004). Chronic inhalation of 4 mg Mo/m^3 as molybdenum trioxide for 4 years was associated with pneumonoconiosis (Vyskocil and Viau, 1999; European Commission, 2000). In addition to respiratory effects, increased blood uric acid concentrations and gout-like symptoms have been reported among workers exposed to molybdenum (Vyskocil and Viau, 1999). Walravens et al. (1979) observed biochemical changes in roasting plant workers exposed for short periods to concentrations of 1.31–6.25 mg/m^3, namely increased serum

[23] Technical Dossier/Section III/Reference 3.3.02.

ceruloplasmin and, to a less extent, of serum uric acid as well as increased plasma and urinary levels of molybdenum.

Some EU countries have established long-term and, in some cases, also short-term exposure limits for molybdenum in the workplace. The more conservative long-term and short-term exposure limits have been established by Germany in 2010 and correspond to 0.5 mg/m^3 and 10 mg/m^3, respectively (Sosiaali- ja terveysministeriö, 2009; Direktoratet for Arbeidstilsynet, 2010; Institut für Arbeitsschutz der Deutschen Gesetzlichen Unfallversicherung, 2010). Considering the available toxicological information, the FEEDAP Panel retains that these limits are also appropriate to evaluate whether the use of sodium molybdate as a feed additive may pose a risk to users.

Assuming the highest parameters for inhalation exposure of users (dusting potential 3.03 g/m^3 with 62.80% represented by particles with diameter lower than 10 μm) and assuming, in the absence of data, that molybdenum make up to approximately 40% of the respirable particles, the resulting concentration in the respirable dust from the additive is 0.8 mg Mo/m^3. Whereas this value is slightly higher than the long-term exposure limit, in accordance with a number of previous opinions the FEEDAP Panel recognises that inhalation exposure of users handling feed additives has a much lower duration during the day than factory workers handling molybdenum compounds and, moreover, it is not continuous. Therefore the FEEDAP Panel considers the slight (i.e. of less than order of magnitude) calculated exceedance of exposure limits to be of no concern.

The FEEDAP Panel considers that, whereas molybdenum is a potential respiratory toxicant, the available data of the additive under evaluation indicate that the use of the additive in animal nutrition poses no risk by inhalation to users.

3.2.5.2. Effects on skin and eye

No original studies were provided by the applicant. The potential of sodium molybdate to elicit skin and ocular irritation or skin sensitization were briefly reviewed in (European Commission, 2000). When tested in rabbits, sodium molybdate (anhydrous form) elicited evident skin irritation for 24 h after application, albeit the skin lesions reversed within 72 . In an eye irritation test on rabbits, a 20% solution did not increase corneal irritation but caused evident conjunctival redness. Based on these findings, sodium molybdate is considered as a skin and eye irritant. The substance is reported not to elicit skin sensitisation (European Commission, 2000 and references herein).

3.2.5.3. Conclusions on safety for the user

Molybdenum is a potential respiratory toxicant; however, the available data indicate that the use of the sodium molybdate under evaluation in animal nutrition poses no risk by inhalation to users. The additive is a skin and eye irritant, but it is not considered as a skin sensitiser.

3.2.6. Safety for the environment

Based on the calculation method provided in the technical guidance for assessing the safety of feed additives for the environment (EFSA, 2008a), the highest theoretical addition of molybdenum in soil (predicted environmental concentration in soil (PEC_{soil})) from animal feeds (lambs) after a 1-year application of manure assuming that 100% of a dose will be excreted, is 53 μg/kg soil. This value exceeds the threshold of 10 μg/kg established in the technical guidance. However, considering that (i) the median content of molybdenum in European soil (i.e. 0.52 mg/kg in subsoil and 0.62 mg/kg in topsoil; detailed information in Appendix A) is over one order of magnitude higher than this value, and (ii) background molybdenum concentrations in feedstuffs across Europe can be even higher than the maximum proposed level of molybdenum supplementation, the FEEDAP Panel considers that the use of molybdenum in sheep feed at 2.5 mg/kg is not expected to pose a risk to the soil compartment. Using the same reasoning, there would also be no concern for the ground or surface water compartments resulting from this application.

3.2.6.1. Conclusions on safety for the environment

The use of sodium molybdate as a feed additive in sheep up to maximum of 2.5 mg of Mo/kg feed poses no concerns for the safety for the environment.

3.3. Efficacy

As previously stated, molybdenum nutritional requirements are relatively low in all animal species and background molybdenum concentrations in feedstuffs are enough to maintain animal health and production; consequently, molybdenum does not need to be added to practical diets (NRC, 2007). However, the antagonistic interaction between copper–sulfur–molybdenum in ruminants makes that, in a few areas where the Cu:Mo ratios in soils and forages may pose a risk to animal health, molybdenum supplementation in the diet may prevent copper toxicity in sheep. Moreover, pasture type and environmental conditions may favour accumulation of copper by the animal even though the copper content may be in the normal range. An example is pasture with a high content of clover (*Trifolium subterraneum*) in which the molybdenum content rarely exceeds 0.1–0.2 mg/kg DM (Todd, 1969). Cereal-rich diets are potentially low in sulfur and molybdenum (Todd, 1972): this may be an important factor in the well-known susceptibility of housed sheep to chronic copper poisoning. Supplemental molybdate compounds, along with sulfate, have been used to prevent copper poisoning in sheep in these instances (MacLachlan and Johnston, 1982; Olson et al., 1984; Humphries et al., 1986). The most common treatment is to give a drench daily containing 50–100 mg of ammonium molybdate and 0.5–1.0 g of sodium sulfate per animal for 3 weeks (Berger and Cunha, 2006). The mechanisms underlying the Cu:Mo interactions in ruminants have been extensively described in the literature (see, e.g. Gould and Kendall, 2011).

3.3.1. Studies on molybdenum efficacy

The FEEDAP Panel asked the applicant to support the efficacy of the additive and molybdenum requirements data with a literature search.[20] For details on how the literature search was performed, see Section 3.2.3. Safety for the target species. The applicant selected 10 references, but some of them could not be further considered because of different reasons (e.g. information from a commercial Veterinary Manual; generic papers for trace minerals for animals; use of trace elements in water for drinking, which is not matter of the application). Only one paper (described below) could be considered partly relevant to the application and was, therefore, examined in more detail. The FEEDAP Panel notes that most of the papers submitted are relatively old and that all of them considered copper supplementation levels higher than those permitted by the EU Regulations.

Gooneratne et al. (1989b) analysed the profiles of ^{67}Cu in blood, bile, urine and faeces from ^{67}Cu- primed lambs and measured the effect of ^{99}Mo-labelled tetrathiomolybdate on the metabolism of recently stored tissue ^{67}Cu. Four lambs (17–22 kg) were fed with 5 or 35 mg Cu/kg DM. They were primed intravenously with ^{67}Cu and challenged 27 h later with ^{99}Mo-labelled tetrathiomolybdate (either intravenously or intraduodenally). The copper and molybdenum profiles were measured in blood, bile, urine and faeces. The level of copper in feed and the route of molybdenum administration affected the copper concentrations in blood, bile and urine. Administration of molybdenum resulted in the immediate release of copper from storage compartments in the body to blood circulation. Biliary and urinary copper excretion due to molybdenum was rapid and maximal within 24 h of injection. The data showed the metabolic pattern by which molybdenum may protect ovines from copper excess.

The FEEDAP Panel recognises that the extensive literature supports the protective effect of molybdenum supplementation toward copper toxicity in ruminants exposed to high dietary copper concentrations (NRC, 1985; Suttle, 2010). The mode of action through which molybdenum affects copper metabolism is nearly exclusive to ruminants on practical conditions and has been described in the chapters on ADME and target animal safety. The complex non-competitive interaction Cu–S–Mo makes that it is unfeasible to give precise 'requirement' values of molybdenum for ruminants under field conditions, since the copper and sulfur content of feed are variable (Suttle, 2010; Tallkvist and Oskarsson, 2015). Molybdenum deficiency per se seldom occurs, if ever, in sheep under typical feeding conditions in the EU; this is due to low basal requirements ($<$ 0.1 mg/kg DM) and to the natural, albeit very variable, presence of molybdenum in most feed materials. Indeed, the use of molybdenum supplementation in sheep farming is mostly associated to its beneficial protective effect toward copper excess, to which ruminants, including sheep, are highly susceptible. Based on Berger and Cunha (2006) and Suttle (2010) the Cu:Mo ratio in the diet is the best tool to estimate whether molybdenum supplementation is required: ratios in the 3–10 range will generally prevent copper toxicity, also taking into account the variable breed susceptibility to copper excess.

3.3.1.1. Conclusions on efficacy for sheep

The FEEDAP Panel recognises that molybdenum does not need to be added to diets to cover the nutritional needs of molybdenum of sheep. Molybdenum supplementation in sheep feed is considered effective in order to guarantee an adequate balance with copper, when the Cu:Mo ratio in the diet is in the range 3–10.

3.4. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[24] and Good Manufacturing Practice.

4. Conclusions

Molybdenum requirements, deficiency and toxicity in ruminants can only be assessed when considering its interactions with other dietary constituents, mainly copper and sulfur. The FEEDAP Panel considers therefore not possible to establish an absolute figure for a dietary molybdenum concentration which is equally safe for sheep and effective in preventing copper toxicity.

The FEEDAP Panel concludes, considering (i) the key parameter to ensure the safety of molybdenum supplementation is the optimal Cu:Mo ratio, which in sheep is in the range of 3–10, and (ii) the highest total copper level authorised in complete feeds for sheep is 15 mg/kg, that 2.5 mg total Mo/kg complete feed is safe for sheep.

Molybdenum would not accumulate in edible tissues or products of sheep fed molybdenum supplemented diets up to the upper maximum level of 2.5 mg/kg complete feed. The consumption of sheep-derived foods would not lead to an intake above the UL (0.01 mg/kg bw) even in high consumers. The FEEDAP Panel considers therefore that the use of sodium molybdate as a feed additive in sheep at the currently maximum authorised level in feed of 2.5 mg Mo/kg is safe for consumers.

Molybdenum is a potential respiratory toxicant; the available data indicate that the use of the sodium molybdate under evaluation in animal nutrition poses no risk by inhalation to users. The additive is a skin and eye irritant, but it is not considered as a skin sensitiser.

The use of sodium molybdate as a feed additive in sheep up to maximum of 2.5 mg of Mo/kg feed poses no concerns for the safety for the environment.

The FEEDAP Panel recognises that molybdenum does not need to be added to diets to cover the nutritional needs of molybdenum of sheep. Molybdenum supplementation in sheep feed is considered effective in order to guarantee an adequate balance with copper, when the Cu:Mo ratio in the diet is in the range 3–10.

Documentation provided to EFSA

1) Dossier Molybdenum – Mo (E7) in the form of Sodium Molybdate, dihydrate. November 2010. Submitted by Trouw Nutrition International B.V.
2) Dossier Molybdenum – Mo (E7) in the form of Sodium Molybdate, dihydrate. Supplementary information. February 2017. Submitted by Trouw Nutrition International B.V.
3) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for Sodium molybdate, dihydrate.
4) Comments from Member States.

Chronology

Date	Event
3/11/2010	Dossier received by EFSA
3/12/2015	Reception mandate from the European Commission
25/1/2016	Application validated by EFSA – Start of the scientific assessment
14/4/2016	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation, safety for target species, safety for the consumer and efficacy*
25/4/2016	Comments received from Member States
28/4/2016	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
14/2/2017	Reception of supplementary information from the applicant - Scientific assessment re-started

[24] Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

Date	Event
24/2/2017	Reception of information from the EC on the request from the applicant to limit the target species to sheep
23/1/2019	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

AAS	atomic absorption spectrometry
ADME	Absorption, distribution, metabolism and excretion
AES	atomic emission spectrometry
AI	adequate intake
ANS	EFSA Panel on Additives and Nutrient Sources added to Food
BW	body weight
CAS	Chemical Abstracts Service
DM	dry matter
DRV	Dietary reference value
EC-No	European Community number
EEC	European Economic Community
EURL	European Union Reference Laboratory
EVM	Expert group on vitamins and minerals
F-TEQ	dibenzofuran-toxic equivalents
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
IUPAC	International Union of Pure and Applied Chemistry
ICP-MS	Inductively coupled plasma mass spectrometry
IPCS	International Programme on Chemical Safety
MTL	maximum tolerable levels
NDA	EFSA Panel on dietetic products, nutrition and allergies
NOAEL	no observed adverse effect level
NRC	National Research Council
NTP	National Toxicology Program
PCDD/F	polychlorinated dibenzodioxin/dibenzofuran
PEC_{soil}	predicted environmental concentration in soil
RSDr	relative standard deviation for *repeatability*
RSDR	relative standard deviation for *reproducibility*
SCF	Scientific Committee on Food
SE	Standard error
UL	Upper intake tolerable level
TDI	Tolerable Daily Intake
WHO	World Health Organization

Appendix A – Background content of molybdenum in soils and feed materials

Molybdenum concentrations in soils

Molybdenum has a crustal abundance of 1.2 mg/kg (Mielke, 1979). Soil molybdenum concentrations vary from 0.1 to 20 mg/kg DM with sandy soils at the low range and those of marine origin at the high extreme. Approximately 10% of soil molybdenum is normally extractable, but the proportion rises with the soil pH (Kabata-Pendias, 2010; Tallkvist and Oskarsson, 2015).

The most comprehensive data of molybdenum concentrations in soils in Europe has been produced within the Geochemical Atlas of Europe (FOREGS survey). The median molybdenum content is 0.52 mg/kg in subsoil and 0.62 mg/kg in topsoil; values range from < 0.1 to 17.2 mg/kg in subsoil and up to 21.3 mg/kg in topsoils. The average ratio topsoil/subsoil is 1.105. Low molybdenum values in subsoil (< 0.32 mg/kg) occur in central Finland, in the glacial drift area from the Netherlands to Poland and Lithuania, in eastern Hungary, south-western France, and small areas in Portugal, Spain and Greece with different geological substrates. High molybdenum values (> 0.91 mg/kg) are found in subsoil in northern Norway and north-west Sweden, most of central Ireland, southern Britain, the French Ardennes, northern Massif Central, the central Alps and northern Appenines, soil on limestone in Slovenia–Croatia (known enrichment in shaly parts of Mesozoic limestone), south-western Austria, southern Italy and Sicily. Single point anomalies occur in north-west Slovakia (Sb-Mo and Cu mineralisation), northern Portugal (WMo mineralisation) and the western Pyrenees (Silurian black shale). A point anomaly in Extremadura (western Spain) is associated with molybdenum bearing veins disseminated in leucogranite.

Molybdenum concentrations in feed materials

Background molybdenum concentrations in feed ingredients mostly reflect environmental conditions. In plants, mean molybdenum concentrations range from 0.33 to 1.5 mg/kg DM in grasses and from 0.73 to 2.3 mg/kg in legumes from different countries. However, depending on the type of soil (i.e. peats, calcareous sands) molybdenum concentrations may be up to 7 mg/kg in grasses and 20 mg/kg in legumes (Kabata-Pendias, 2010).

Across Europe, mean molybdenum concentrations in feed materials used in ruminant nutrition is normally below 1 mg/kg DM, except for oilseed meals (2–4 mg/kg) and lucerne and grass (1.5–3 mg/kg) (Van Paemel et al., 2010; see also Annex B).

In most feed materials, background levels of molybdenum are < 1 mg/kg DM. Exceptions are represented by legumes (e.g. alfalfa), data from Bulgaria and, outside Europe, from Canada (see Table A.1). Background values of molybdenum in typical feeds are presented in Annex C.

The most relevant data in feed materials and vegetables are summarised in the table below.

Table A.1: Concentration of molybdenum in feed materials

Feed	Mo (mg/kg DM)	Reference Country
Pasture grass	0.3	Nehring et al. (1970)
Red clover	0.2–0.5	Germany
Alfalfa	0.3	
Corn silage	0.4	
Cereal straw	0.1–0.6	
Potatoes	0.2	
Sugar beets	0.1	
Barley	0.4	
Corn	0.2	
Wheat	0.2	
Rape seed extracted meal	0.6	
Soy bean extracted meal	4.0	
Legumes	1.8	Miltimore and Masch (1971)
Grass	2.0	Canada
Oat forage	1.5	
Corn silage	0.9	
Cereals	1.0	

Feed	Mo (mg/kg DM)	Reference Country
Grasses	0.2–0.8	Mills and Davis (1987)
Legumes	0.5–1.5	USA
Straws	0.2–0.5	
Cereals	0.2–0.5	
Vegetable protein concentrates	0.5–2.0	
Grass		Mills and Davis (1987)
(soil-pH: 5.5)	1.1	USA
(soil pH: 7.5)	5.2	
Head of lettuce		Falke (1988)
no Mo	0.11	Germany
+ 0.5 kg Mo/ha	0.15	
+ 1.0 kg Mo/ha	0.35	
+ 2.0 kg Mo/ha	0.56	
+ 8.0 kg Mo/ha	1.4	
+ 32 kg Mo/ha	2.6	
Alfalfa (influence of soil)		Regius and Anke (1989)
Löss-soil	0.84–1.3	Hungary
Forest soils	0.63–0.82	
Aluvial soils	1.3–2.5	
Sandy soils	0.4–1.0	
Acid soils (Szikes, Moor, Torf)	2.6–3.8	
Alfalfa (influence of Crude protein content)		
< 18% CP of DM	1.3 ± 0.3	
18–19%	1.7 ± 0.3	
19–20%	1.8 ± 0.4	
20–21%	2.1 ± 0.6	
21–22%	2.4 ± 0.6	
> 22%	3.1 ± 0.5	
Maize meal	0.092	Anke et al. (1993)
Wheat meal	0.175	Germany
Rice	0.357	
Oat meal	0.376	
Peas	2.353	
Lentils	4.173	
Beans	6.090	
Grass	0.19	Kleczkowski et al. (1995)
Protein concentrate	0.43	Poland
Straw	0.10	
Apples	0.16	Holzinger et al. (1996)
Carrots	0.25	Germany
Potatoes	0.73	
Oats	0.36	
Dried milk	0.271	Holzinger et al. (1998) Germany
		Kabata-Pendias (2000)
Barley	0.29	Canada
Oats	0.41	
Wheat	0.18	
Barley (Spring wheat)	0.29–0.41	Czech Republic
Oats	0.59–0.84	
Wheat (Winter wheat)	0.18–0.42	
Barley	0.19–0.49	Finland
Oats	0.21–0.59	
Rye	0.23–0.57	
Wheat (Winter wheat)	0.14–0.38	
Barley	0.02–0.59	Norway
Wheat	0.07–1.09	

Feed	Mo (mg/kg DM)	Reference Country
Wheat (Winter wheat)	0.02–0.60	Poland
Barley	0.54–1.00	Sweden
Barley	0.58–2.40	USA
Oats	0.28–1.90	
Wheat (Spring wheat)	0.08–1.10	
Wheat (Winter wheat)	0.40–1.10	
Rice	0.18–3.05	
Barley	0.69–0.75	Russia
Oats	0.42–0.62	
Rye	0.12–1.31	
Wheat	0.26–1.31	
From areas where Mo toxicity in grazing animals Was Not observed:		
Grasses	0.4–0.8	Canada
	0.14–0.80	Finland
	0.23–0.91	
	0.08–1.04	Germany
	0.42–0.84	
	0.5–4.0(2)	Great Britain
	0.25–1.47	
	0.18–0.77	Ireland
	0.04–3.05	Japan
	< 0.02–1.68	Poland
	0.2–4.8	Sweden
	0.5–2.0(1)	USA
Legumes	0.04–0.32	Bulgaria
	0.02–1.30	Finland
	0.21–0.50	Germany
	0.28–0.52	Ireland
	0.01–3.64	Japan
	0.02–3.56	Poland
	0.3–20.5	Sweden
	< 0.7–15.0(3)	USA
	0.01–3.46	
	1.31–3.61	Russia
From areas where Mo toxicity in grazing animals Was observed:		
Grasses	0.6–17.0	Canada
	2.4–12.0	
	0.1–7.2	Great Britain
	1–234(2)	Sweden
	0.7–6.8	USA
	10–50(2)	Russia
Legumes	1.0–20.0	Canada
	4.8–6.0	
	18.9–39.6	USA
Pasture grass (Gneiss soil)		Kirov et al. (2002)
May	2.02	Bulgaria
June	1.37	
July	0.79	
August	1.05	
September	2.01	
Pasture grass (Syenit soil)		
May	0.55	
June	1.14	
July	1.74	

Feed	Mo (mg/kg DM)	Reference Country
August September	1.03 0.66	
Grass (Panchmahal District, India)	< 1 mg	Garg et al. (2003) India
Various feeds	< 0.77	Garg et al. (2005) Germany
Barley Oats Maize Rice Rye Sorghum Wheat Milk powder (whole milk) Dried skimmed milk	0.43 (0.32–0.52) 0.70 (0.1–1.5) 0.55 (0.50–0.58) 0.31 (0.20–1.0) 0.46 1.7 (0–1.7) 0.35 (0.34–0.40) 0.29 0.29	Souci et al. (2008)[(1)] Germany
Various feeds (Bharapur district; India) Wheat straw Sorghum straw Wheat Barley Sorghum	 0.29 0.15 0.06 0.09 0.12	Garg et al. (2008) India
Various feeds (Ajmer district, India) Wheat straw Sorghum straw Maize straw Local grasses Alfalfa Barley	 0.89 0.59 1.02 0.90 2.46 1.87	Garg et al. (2009) India

(1): Values on 88% DM.
(2): Pasture herbage.
(3): Calculated on ash weight basis.

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Appendix B – Molybdenum toxicity in ruminants

The most comprehensive review of molybdenum toxicity in ruminants has been carried out by the National Research Council (NRC, 2005). The FEEDAP Panel extracted relevant text from this review which is reported below.

'When ruminants ingest moderate to high levels of molybdenum from pasture, a concomitant high intake of sulfur induces copper deficiency (Ward, 1978). The disorder was initially attributed to the formation of insoluble copper-molybdenum-sulfur complexes (mono-, di, tri-, and tetra-thiomolybdates) in the rumen (Gooneratne et al., 1989; Suttle, 1991). However, Allen and Gawthorne (1987) argued for the importance of an association of thiomolybdates and copper with proteins in the solid digesta, suggesting that the molybdenum-copper antagonism was not a direct action, but a consequence of molybdenum affinity for sulfide generated within the rumen. After ruminal administration of ^{99}Mo-labeled compounds, tri- and tetrathiomolybdates were found in the solid phase of ruminal, duodenal, and ileal digesta, whereas di- and tri-thiomolybdates were detected in the plasma of sheep (Price et al., 1987). Likely, the inhibition of copper absorption was mediated by tri- and tetra-thiomolybdates, and the postabsorptive effect on copper metabolism was exerted by di- and trithiomolybdates. In summary, absorbed thiomolybdates may affect copper metabolism in the following ways: (1) enhance biliary excretion of copper from liver stores; (2) reduce transport of available copper for biochemical synthesis by binding copper to plasma albumin; and (3) remove copper from cupro-enzymes (Suttle, 1991; Spears, 2003). Obviously, the presence of the sulfide-generating microbes in the rumen renders cattle and sheep susceptible to the molybdenum-copper-sulfur imbalance.

Anorexia and body weight loss are typical signs of chronic molybdenosis in cattle. When these animals graze on "teart" pasture containing 20–100 mg Mo/kg dry matter (normal: 3–5 mg of Mo/kg, Underwood and Suttle, 1999) scours may occur within 24 h (Lloyd et al., 1976). Prolonged high molybdenum intake in cattle also produced anemia, achromotrichia, posterior weakness, skeletal deformities and reproductive abnormalities (Venugopal and Luckey, 1978). These signs were shown in early studies with various types of cattle fed molybdenum from 6.2 to 400 mg/kg of diets (NRC, 1980). Severe molybdenum toxicosis in weanling heifers fed 100 mg Mo (as sodium molybdate)/kg of diet for 336 days was manifested as scouring, achromotrichia, anemia, weight loss, and 31% mortality within 2 weeks after the study began (Lesperance et al., 1985). Secondary copper deficiency was induced in beef heifers by feeding 7 to 16 mg Mo (as sodium molybdate)/kg diet in the presence of 0.3% of sulfur and 3 to 6 mg of Cu/kg (Arthington et al., 1996a,b); the molybdenum-supplemented animals also showed decreased plasma copper and ceruloplasmin and increased plasma fibrinogen and blood neutrophil numbers. When 5-week-old Holstein calves were given water containing 1, 10, or 50 mg Mo (as ammonium molybdate)/L for 21 days, liver copper content was reduced in the calves receiving the highest level of molybdenum (Kincaid, 1980); the calculated proportion of plasma copper as ceruloplasmin was reduced from 61% to 43% with increases in water molybdenum levels, indicating a reduced copper uptake by tissues from the plasma. Finally, molybdenum concentrations as low as 5 mg Mo/kg may be detrimental to copper-deficient cattle (Gengelbach et al., 1997) and has been demonstrated to cause copper depletion in heifers when Cu:Mo ratios are not adequate (Bremner et al., 1987). Based on the responses of growth, liver copper concentration, and plasma copper distribution, Kincaid (1980) suggested the minimal toxic concentration of molybdenum in drinking water for calves was between 10 and 50 mg/L, and the critical copper:molybdenum ratio is < 0.5 when the animals were given diets containing 13 mg Cu/kg and 0.29 percent sulfur. In a semi-purified diet that contained 1.1 mg Cu and 1.1 mg Mo/kg of dry matter for calves, supplementing 5 mg Mo (as sodium molybdate)/kg depressed humoral immune responses and erythrocyte superoxide dismutase activity, compared with those supplemented with 10 mg of Cu/kg (Gengelbach and Spears, 1998). Supplementing 5 mg Mo (as sodium molybdate)/kg of diet to 7-month old steers for 245 days reduced plasma copper and ceruloplasmin concentrations, and erythrocyte superoxide dismutase activity in the non-copper-supplemented steers, but had no effect on performance or carcass quality (Ward and Spears, 1997). Steers exhibited no further changes in copper status when dietary molybdenum was increased from 5 to 10 mg (as sodium molybdate)/kg of diet in the presence of 2.7 g S/kg diet (Gengelbach, 1994). Compared with the controls, heifers or steers showed no adverse response after grazing bahia grass pasture treated with high molybdenum biosolids (molybdenum loads from 0.27 to 2.56 kg/Ha) for 6 months (Tiffany et al., 2000, 2002).

Clinical signs of chronic molybdenum toxicosis in sheep are essentially secondary hypocuprosis: reduced crimp and pigmentation of wool, anemia, alopecia, and depressed weight gain. When molybdenum is given at very high concentrations in the diet, the level necessary to produce symptoms in sheep is higher than for cattle, but not precisely determined; in one study in sheep fed 120 mg Mo daily for 29 months no clinical signs of copper deficiency (apart from the condition of the wool) were found (Bingley, 1974). When sheep were fed a basal diet containing 1.0 g sulfur and 0.5 mg Mo/kg diet, supplementing 4 mg Mo and 3 g S per kg diet reduced copper bioavailability by 40–70%, but the addition of only molybdenum had no effect at all (Suttle, 1975). Increasing molybdenum (as ammonium molybdate) from 0.4 to 8.4 mg/kg DM in the diets for wethers resulted in reduced daily gains, poor feed efficiency, and decreased solubility of copper and molybdenum in the rumen, along with increased liver and kidney molybdenum contents (Ivan and Veira, 1985); there was no effect on liver copper content. Supplementing molybdenum at 10–40 mg Mo (as tetrathiomolybdate)/kg DM into a copper and chromium deficient diet for male goats exacerbated the copper deficiency (Aupperle et al., 2001). In contrast, Anke et al. (1985a,b) reported that goats tolerated diets with 1 g of Mo/kg diet, and they suggested that this high tolerance was not due to insufficient molybdenum absorption or related to copper metabolism'.

Some limited and old studies suggest that molybdenum may be a reproductive toxicant in ruminants. In this context, the Scientific Committee on Food (European Commission, 2000) reported the following:

'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)'.

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Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Sodium molybdate dihydrate

In the current application authorisation is sought under article 10(2) for *sodium molybdate* under the category/functional group (3b) "nutritional additives"/"compounds of trace elements", according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the *feed additive* for all categories and species.

The *feed additive* is white crystalline powder, marketed in the form of sodium molybdate dihydrate (Na_2MoO_4 $2H_2O$), containing a minimum of 37% molybdenum. The *feed additive* is intended to be incorporated into *feedingstuffs* through *premixtures* with a maximum recommended level of 2.5 mg *total molybdenum*/kg complete *feedingstuffs.*

For the characterisation of the *feed additive* the Applicant did not propose any method. However, the EURL identified the European Pharmacopoeia monograph 1565, where: - identification is based on "loss on drying" and specific reactions of molybdate and sodium ions; while - quantitative assay is based on titration with lead nitrate in the presence of hexamethylenetetramine and nitric acid till the endpoint determined by 4-(2-pyridylazo) resorcinol monosodium salt as an indicator.

For the quantification of *total sodium* in the *feed additive,* the EURL identified two internationally recognised ring-trial validated CEN methods: (i) EN 15510, based on Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES); and (ii) EN ISO 6869, based on Atomic Absorption Spectrometry (AAS).

For the *quantification* of *total molybdenum* in the *feed additive, premixtures* and *feedingstuffs* the Applicant submitted the internationally recognised ring-trial validated CEN method (EN 15510) based on inductively coupled plasma atomic emission spectroscopy (ICP-AES). The Applicant applied the ICP-AES method to quantify *total molybdenum* in five batches of the *feed additive* and reported a relative standard deviation for *repeatability* (RSDr) of 1.9% for samples containing 39% *total molybdenum.* The following performance characteristics were reported in the EN 15510 standard for *premixtures* and *feedingstuffs* samples containing from 1.06 to 16700 mg/kg *total molybdenum:* RSDr ranging from 3.1 to 16%; and a relative standard deviation for *reproducibility* (RSDR) ranging from 11 to 32%.

Based on the data available the EURL recommends for official control the above mentioned ring-trial validated CEN methods for the quantification of *total molybdenum* and *total sodium* in the *feed additive; premixtures* and *feedingstuffs* (only *total molybdenum).*

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.

Annex B – Extract of Van Paemel et al. (2010) on Molybdenum concentration in feed materials

Annex 4. Molybdenum concentration in feed materials according to CVB[1] and INRA[2] feed composition tables[3]

CVB	
COMPOUND FEED INGREDIENTS	mg/kg
Potatoes dried	
Potato crisps	
Potato prot ASH<10	
Potato prot ASH>10	
Potato starch dried	
Potato sta heat tr	
Potato pulp CP<95	
Potato pulp CP>95	
Potatoes sweet dried	
Bone meal	
Brewers' grains dr	
Brewers' yeast dried	1.1
Sugarb pulp SUG<100	0.4
Sugarb p SUG100-150	0.3
Sugarb p SUG150-200	
Sugarb pulp SUG>200	
Biscuits CFAT<120	
Biscuits CFAT>120	
Blood meal spray dr	
Buckwheat	
Beans phas heat tr	
Bread meal	
Casein	
Chicory pulp dried	0.8
Citrus pulp dried	
Meat meal Dutch	
Meat meal CFAT<100	0.6
Meat meal CFAT>100	
Peas	3
Barley	0.2
Barley feed h grade	
Barley mill byprod	
Grass meal CP<140	2.2
Grass meal CP140-160	2.2
Grass meal CP160-200	2.2
Grass meal CP>200	2.2
Grass seeds	
Peanuts wtht shell	
Peanuts with shell	
Peanut exp wtht sh	2.1
Peanut exp p with sh	
Peanut exp with sh	
Peanut extr wtht sh	
Peanut extr with sh	
Oats grain	0.7
Oats grain peeled	0.2
Oats husk meal	
Oats mill fd h grade	
Hempseed	
Carob	

INRA	Mean	St. Dev.
	mg/kg	
CEREALS		
Barley	0.44	
Maize	0.41	
Oats	0.83	
Oats groats	0.19	
Rice, brown	0.75	
Rye	0.55	
Sorghum	1	
Triticale	0.44	
Wheat, durum		
Wheat, soft	0.46	
WHEAT BY-PRODUCTS		
Wheat bran	1.4	
Wheat middlings	2	
Wheat shorts	0.07	
Wheat feed flour		
Wheat bran, durum		
Wheat middlings, durum		
Wheat distillers' grains, starch <7%		
Wheat distillers' grains, starch >7%		
Wheat gluten feed, starch 25%		
Wheat gluten feed, starch 28%		
MAIZE BY-PRODUCTS		
Corn distillers	1.7	
Corn gluten feed	1.6	
Corn gluten meal	0.82	
Maize bran		
Maize feed flour		
Maize germ meal, expeller		
Maize germ meal, solvent extracted		
Hominy feed	1.1	
OTHER CEREAL BY-PRODUCTS		
Barley rootlets, dried	1.1	
Brewers' dried grains	1.3	
Rice bran, extracted		
Rice bran, full fat	1.6	
Rice, broken	0.08	
LEGUME AND OIL SEEDS		
Chickpea		
Cottonseed, full fat	1.5	
Faba bean, coloured flowers	0.63	
Faba bean, white flowers	0.63	
Linseed, full fat	0.2	
Lupin, blue	2	
Lupin, white	2	
Pea	2	
Rapeseed, full fat		
Soybean, full fat, extruded	4	
Soybean, full fat, toasted	4	
Sunflower seed, full fat	1.7	

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CVB	
COMPOUND FEED INGREDIENTS	mg/kg
Canaryseed	
Greaves	
Cottonseed wtht husk	
Cottonseed with husk	
Cottons exp wtht h	
Cottons exp p with h	
Cottons exp with h	
Cottons extr wtht h	
Cotts extr p with h	
Cottons extr with h	
Coconut exp CFAT<100	
Coconut exp CFAT>100	
Coconut extr	0.6
Linseed	0.2
Linseed exp	0.5
Linseed extr	0.7
Lentils	
Lupins CP<335	
Lupins CP>335	
Alf meal CP<140	
Alf meal CP140-160	
Alf meal CP160-180	0.5
Alf meal CP>180	
Poppyseed	
Macoya fruit exp	
Maize	0.3
Maize chem-h treated	0.3
Maize gluten meal	0.6
Maize glfeed CP<200	
Maize glfd CP200-230	
Maize glfeed CP>230	
Maize germ meal extr	
Maize germ m fd exp	
Maize germ m fd extr	
Dist grains and sol	
Maize feedflour	
Maize feed meal	
Maize feed meal extr	
Maize bran	
Maize starch	
Sugarbeet molasses	0.2
Sugarc mol SUG<475	
Sugarc mol SUG>475	
Milk powder skimmed	
Milk powder whole	
Millet	

INRA	Mean	St. Dev.
	mg/kg	
OIL SEED MEALS		
Cocoa meal, extracted		
Copra meal, expeller	0.61	
Cottonseed meal, crude fibre 7-14%	0.8	
Cottonseed meal, crude fibre 14-20%	3	
Grapeseed oil meal, solvent extracted		
Groundnut meal, detoxified, crude fibre < 9%	1.7	
Groundnut meal, detoxified, crude fibre > 9%	2	
Linseed meal, expeller	0.52	
Linseed meal, solvent extracted	1	
Palm kernel meal, expeller	0.4	
Rapeseed meal	1.6	
Sesame meal, expeller	1.9	0.1
Soybean meal, 46		
Soybean meal, 48	4	
Soybean meal, 50	3	
Sunflower meal, partially decorticated	1.6	
Sunflower meal, undecorticated	0.7	
STARCH, ROOTS AND TUBERS		
Cassava, starch 67%	0.05	
Cassava, starch 72%		
Maize starch		
Potato tuber, dried	1.2	
Sweet potato, dried		
OTHER PLANT BY-PRODUCTS		
Alfalfa protein concentrate		
Beet pulp, dried	0.67	
Beet pulp dried, molasses added	0.29	
Beet pulp, pressed		
Brewers' yeast, dried	1.1	
Buckwheat hulls		
Carob pod meal		
Citrus pulp, dried	0.19	
Cocoa hulls		
Grape marc, dried		
Grape seeds		
Liquid potato feed		
Molasses, beet	0.26	
Molasses, sugarcane	1.3	
Potato protein concentrate		
Potato pulp, dried		
Soybean hulls	1.2	
Vinasse, different origins		
Vinasse, from the production of glutamic acid		
Vinasse, from yeast production		
Wheat distillers' grains		

CVB	
COMPOUND FEED INGREDIENTS	mg/kg
Millet pearlmillet	
Malt culms CP<200	
Malt culms CP>200	
Nigerseed	
Horsebeans	0.4
Horsebeans white	0.4
Palm kernels	
Palm kern exp CF<180	0.5
Palm kern exp CF>180	0.5
Palm kernel extr	
Rapeseed	
Rapeseed exp	1.1
Rapeseed extr CP<380	0.8
Rapeseed extr CP>380	
Rapes meal Mervobest	0.8
Rice wtht hulls	
Rice with hulls	
Rice husk meal	
Rice bran meal extr	
Rice feed m ASH<90	
Rice feed m ASH>90	
Rye	1.1
Rye middlings	
Safflowerseed	
Safflower meal extr	
Sesameseed	
Sesameseed exp	
Semameseed meal extr	2.8
Soybeans heat tr	
Soybeans not heat tr	
Soybean hulls CF<320	
Soyb hulls CF320-360	
Soybean hulls CF>360	
Soybean exp	
Soybm CF<45 CP<480	3.8
Soybm CF<45 CP>480	3.8
Soybm CF45-70 CP<450	3.9
Soybm CF45-70 CP>450	3.9
Soyb meal CF>70	3.8
Soyb meal Mervobest	3.8
Soyb meal Rumi S	3.5
Sorghum	
Sorghum gluten meal	
Sugar	
Tapioca STA 575-625	
Tapioca STA 625-675	
Tapioca STA 675-725	
Tapioca starch	

INRA	Mean	St. Dev.
	mg/kg	
DEHYDRATED FORAGES		
Alfalfa, dehydrated, protein < 16% dry matter	1.4	
Alfalfa, dehydrated, protein 17-18% dry matter	1.4	
Alfalfa, dehydrated, protein 18-19% dry matter	1.4	
Alfalfa, dehydrated, protein 22-25% dry matter	1.4	
Grass, dehydrated	2	
Wheat straw	1.2	
DAIRY PRODUCTS		
Milk powder, skimmed	0.24	
Milk powder, whole	0.4	
Whey powder, acidic	5	
Whey powder, sweet	5	
FISH MEALS AND SOLUBLES		
Fish meal, protein 62%	0.1	
Fish meal, protein 65%	0.21	
Fish meal, protein 70%	0.18	
Fish solubles, condensed, defatted		
Fish solubles, condensed, fat		
OTHER ANIMAL BY-PRODUCTS		
Blood meal	0.21	
Feather meal	0.9	
Meat and bone meal, fat <7.5%	1	
Meat and bone meal, fat >7.5%	1	

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CVB

COMPOUND FEED INGREDIENTS	mg/kg
Wheat	1.1
Wheat gluten meal	
Wheat glutenfeed	
Wheat middlings	0.7
Wheat germ	
Wheat germfeed	
Wheat feedfl CF<35	
Wheat feedfl CF35-55	
Wheat feed meal	
Wheat bran	0.6
Triticale	0.4
Feather meal hydr	
Fat from Animals	
Fats/oils veg h %d	
Fats/oils vegetable	
Vinasse Sugb CP<250	
Vinasse Sugb CP>250	
Fish meal CP<580	
Fish meal CP580-630	
Fish meal CP630-680	
Fish meal CP>680	
Meat bone m CFAT<100	
Meat bone m CFAT>100	
Whey p l lac ASH<210	
Whey p l lac ASH>210	
Whey powder	
Sunflowers deh	
Sunflowers p deh	
Sunflowers w hulls	
Sunfls exp deh	
Sunfls exp p deh	
Sunfls exp w hulls	
Sunfmeal CF<160	
Sunfmeal CF 160-200	
Sunfmeal CF 200-240	
Sunfmeal CF>240	
MOISTURE RICH FEED INGREDIENTS	**mg/kg DM**
Potato juice conc	
Potato pulp pr NL	
Potato pulp pressed	
Potato cut raw	
Potato c CFAT 40-120	
Potato c CFAT120-180	
Potato cut CFAT>180	
Potato p st STA<350	
Pot p st STA350-475	
Pot p st STA475-600	
Potato p st STA>600	
Potato starch solid	
Pot sta STA 500-650	
Pot sta STA 650-775	
Pot sta STA>750	

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CVB	
MOISTURE RICH FEED INGREDIENTS	mg/kg DM
Pot s g STA 300-425	
Pot s g STA 425-550	
Pot s g STA 550-675	
Pot sta gel STA>675	
Brewers gr 22% DM	
Brewers gr 27% DM	
Brewers yeast CP<400	
Brewers y CP400-500	
Brewers yeast CP>500	
Beetp pressed f+sil	0.4
CCM CF<40	0.4
CCM CF 40-60	
CCM CF>60	
Chicory pulp f+sil	0.7
Distillers sol f	
Cheese whey CP<175	
Cheese w CP175-275	
Cheese whey CP>275	
Maize glutenf f+sil	
Maize solubles	
Wheat st FR STAt 300	
Wheat st STAtot 400	
Wheat st STAtot 600	
Carrot peelings st p	
ROUGHAGES AND COMPARABLE PRODUCTS	mg/kg DM
Potatoes fresh	
Potatoes sil	
Potato-peelings sil	
Endive fresh	
Apples fresh	
Gherkin fresh	
Beet leaves fresh	
Beet leaves w p beet	
Beet leaves sil	1.4
Beet rests siliId	
Bean straw (Phas)	
Bean straw (Vicia)	
Chicory leaves fresh	
Chicory leaves sil	
Pea haulm fresh	
Pea haulm sil	
Pea straw	
Whole crop sil(Cer)	1.3
Barley straw	
Grass fr April l y.	2.7
Grass fr April n y.	2.7
Grass fr April h y.	2.7
Grass fr May l y.	2.7
Grass fr May n y.	2.7
Grass fr May h y.	2.7
Grass fr June l y.	2.7
Grass fr June n y.	2.7
Grass fr June h y.	2.7

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CVB	
ROUGHAGES AND COMPARABLE PRODUCTS	mg/kg DM
Grass fr July l y.	2.7
Grass fr July n y.	2.7
Grass fr July h y.	2.7
Grass fr Aug l y.	2.7
Grass fr Aug n y.	2.7
Grass fr Aug h y.	2.7
Grass fr Sept l y.	2.7
Grass fr Sept n y.	2.7
Grass fr Sept h y.	2.7
Grass fr Oct l y.	2.7
Grass fr Oct n y.	2.7
Grass fr Oct h y.	2.7
Grass average	2.7
Grass horse gr past	2.7
Grass horse same fld	2.7
Grass sil May 2000	2.1
Grass sil May 3500	2.1
Grass sil May 5000	2.1
Grass sil June 2000	2.1
Grass sil June 3000	2.1
Grass sil June 4000	2.1
Grass sil Ju-Au 2000	2.1
Grass sil Ju-Au 3000	2.1
Grass sil Ju-Au 4000	2.1
Grass sil Se-Oc 2000	2.1
Grass sil Se-Oc 3000	2.1
Grass sil average	2.1
Grass sil horse fine	2.1
Grass sil horse midd	2.1
Grass sil horse crs	2.1
Grass hay good qual	2.1
Grass hay av qual	2.1
Grass hay poor qual	2.1
Grass hay horse fine	2.1
Grass hay horse midd	2.1
Grass hay horse crs	2.1
Grass bales ad	2.4
Grass seeds straw	2.2
Oat straw	
Clover red fresh	
Clover red silage	2.6
Clover red hay	
Clover red ad	
Clover red straw	
Cucumber fresh	
Winterrape	
Marrowstem	
Cauliflower	
Kale (white-red)	
Brussels sprouts l&s	
Brussels sprouts	
Turnip cabbage	
Beetroot	

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CVB	
ROUGHAGES AND COMPARABLE PRODUCTS	mg/kg DM
Lucerne fresh	
Lucerne silage	
Lucerne hay	
Lucerne (alfalfa) ad	2.2
Maize Cob with leaves silage	
Sweet pepper fresh	
Pears fresh	
Leeks fresh	
Rye straw	
Lettuce fresh	
Green cereals fresh	
Green cereals silage	1.8
Maize fod fr DM<240	0.4
Maize f fr DM240-280	0.4
Maize f fr DM280-320	0.4
Maize fod fr DM 320	0.4
Maize sil DM < 240	0.4
Maize sil DM240-280	0.4
Maize sil DM280-320	0.4
Maize sil DM 320	0.4
Maize (Fodder) ad	0.4
Spinach fresh	
Sugar beets fresh	
Wheat straw	
Tomatoes fresh	
Onions	
Field beans silage	
Fodderbeets dirty	
Fodderbeets cleaned	
Chicory rts not frcd	
Chicory rts frcd cleaned	
Chicory rts frcd dirty	
Carrots	
Sunflower silage	

MINERAL FEEDS[3]	mg/kg
Bone meal (steamed)	
Calcium carbonate	
Diammonium phosphate	
Difluorinated phosphate	
Dicalcium phosphate	
Mono-dicalcium phosphate	
Monoammonium phosphate	
Sodium tripolyphosphate	
Phosphoric acid (75%)	

[1] CVB. 2007. Feed Tables. Produktschap Diervoeding, The Netherlands; [2] INRA. 2004. Tables of composition and nutritional value of feed materials. Wageningen Academic Publishers, The Netherlands & INRA, Paris, France; [3] For mineral feeds element concentrations are from Batal and Dale. 2008. Feedstuffs September 10, p. 16

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Van Paemel M, Dierick N, Janssens G, Fievez V and De Smet S, 2010. Selected trace and ultratrace elements: Biological role, content in feed and requirements in animal nutrition—Elements for risk assessment. Technical Report submitted to EFSA. Available online: http://www.efsa.europa.eu/en/supporting/pub/68e.htm

Annex C – Extract of Van Paemel et al. (2010) on Background values of molybdenum in typical feeds

Annex 5. Background concentration of molybdenum in a representative complete feedingstuff for a list of farm animal categories using CVB[1] and INRA[2] trace element composition tables[3]

	# Feed materials	Mass with element concentration (%)		# Feed materials with element concentration		Element concentration (mg/kg)	
		CVB	INRA	CVB	INRA	CVB	INRA
Piglet Starter I (from weaning)	9	74.1	89.2	5	6	0.603	1.200
Piglet Starter II (complete feed)	20	78.2	77.7	7	8	0.779	0.618
Pig Grower (complete feed)	19	86.1	88.4	7	9	0.730	0.666
Pig Finisher (complete feed)	18	78.3	90.6	6	8	0.556	0.655
Sows, gestating (complete feed)	18	71.5	83.2	6	9	0.362	0.628
Sows, lactating (complete feed)	20	76.1	78.1	7	9	0.661	0.658
Starter Chicks (complete feed)	15	80.4	84.0	4	5	1.259	0.923
Chicken reared for laying (complete feed)	17	72.0	79.5	5	6	0.714	0.559
Layer Phase I (complete feed)	16	64.1	86.5	3	6	0.705	0.908
Layer Phase II (complete feed)	16	56.7	78.5	3	6	0.635	0.862
Broiler Starter (complete feed)	14	79.1	94.1	4	5	1.115	1.425
Broiler Grower (complete feed)	15	81.7	89.2	5	5	1.317	1.276
Broiler Finisher (complete feed)	15	80.3	88.0	4	4	1.402	1.258
Turkey Starter (complete feed)	14	87.8	92.8	3	4	1.952	1.481
Turkey Grower (complete feed)	13	88.2	90.2	3	4	2.028	1.530
Turkey Finisher (complete feed)	11	91.2	91.2	3	3	1.976	1.417
Turkey Breeder (complete feed)	8	80.8	82.8	2	3	0.642	0.645
Duck, grower/finisher (complete feed)	10	92.9	92.9	3	3	1.391	0.947
Geese, grower/finisher (complete feed)	8	97.0	97.0	4	4	1.596	1.265
Calf, milk replacer (complete feed)	10	10.0	30.7	1	1	0.380	1.533
Calf concentrate (complete feed)	17	65.3	86.1	6	10	1.132	1.467
Calf concentrate (complementary feed)	16	30.6	72.2	5	9	0.164	0.935
Cattle concentrate (complete feed)[4]	9	85.9	95.9	6	7	1.295	1.178
Cattle concentrate (complementary feed)	8	79.8	94.1	5	6	0.950	0.826
Dairy cows TMR (based on corn silage)[4]	15	88.5	98.8	7	10	1.086	1.147
Dairy cows TMR (based on grass silage)[4]	15	86.1	97.9	7	10	1.330	1.406
Dairy concentrate (complementary feed)	13	40.4	90.1	5	8	0.582	1.131
Dairy cows mineral feed (min. 40% crude ash)	8	0.0	0.0	0	0	0.000	0.000
Rabbit, breeder (complete feed)	8	51.0	97.0	3	4	0.546	1.483
Rabbit, grower/finisher (complete feed)	14	75.0	95.0	4	6	0.483	1.046
Salmon feed (wet)[4]	4	14.9	70.4	1	2	0.164	0.168
Salmon feed (dry)	6	27.4	79.4	2	3	0.842	0.728
Trout feed (dry)	12	57.9	78.2	2	4	2.122	1.775
Dog food (dry)	12	72.7	81.1	3	5	0.340	0.616
Cat food (dry)	16	17.0	68.1	3	7	0.160	0.567

[1] CVB. 2007. Feed Tables. Productschap Diervoeding, The Netherlands; [2] INRA. 2004. Tables of composition and nutritional value of feed materials. Wageningen Academic Publishers, The Netherlands & INRA, Paris, France; [3] For mineral sources element concentrations were used from Batal and Dale. 2008. Feedstuffs September 10, p. 16; [4] On DM basis

(For more information on the substantiation of the data of the table above, please refer to the report of Van Paemel et al. (2010).

Van Paemel M, Dierick N, Janssens G, Fievez V and De Smet S, 2010. Selected trace and ultratrace elements: biological role, content in feed and requirements in animal nutrition—Elements for risk assessment. Technical Report submitted to EFSA. Available online: http://www.efsa.europa.eu/en/supporting/pub/68e.htm