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Safety and efficacy of zinc chelate of methionine sulfate for all animal species

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Abstract

The additive 'Zinc chelate of methionine sulfate' is zinc chelated with methionine in a molar ratio 1:1, with a minimum zinc content of 19.1%. Owing to the limitations of the tolerance study, it could not be used for the assessment of safety for target animals; therefore, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) cannot conclude on the safety of zinc chelate of methionine sulfate for the target species. No concerns for consumer safety are expected from the use of the zinc chelate of methionine sulfate in animal nutrition when used up to the maximum EU authorised zinc levels in feed. Zinc chelate of methionine sulfate should be considered as a skin and eye irritant, and as a skin sensitiser; it is considered to pose a risk by inhalation to the users. The additive under assessment, zinc chelate of methionine sulfate, is intended to be a substitute for other authorised zinc additives and will not further increase the environmental burden of zinc; therefore, the FEEDAP Panel considers that the use of the additive in animal nutrition would not pose an additional risk for the environment. Based on literature studies and a specific study conducted with the additive under assessment, zinc chelate of methionine sulfate is an available source of zinc for all animal species.

Keywords: nutritional additives, compounds of trace elements, zinc, zinc methionine complex, zinc chelate of methionine sulfate, safety, efficacy

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Norel, S.A.[2] for authorisation of the product zinc chelate of methionine hydrate,[3] when used as a feed additive for all animal species (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 4(1) (authorisation of a feed additive or new use of a 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 18 December 2015.

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 *zinc chelate of methionine sulfate*, when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

The Scientific Committee on Animal Nutrition (SCAN) issued an opinion on the use of zinc in feedingstuffs (EC, 2003a). EFSA issued an opinion on the safety of the chelated forms of zinc with synthetic feed grade glycine (EFSA FEEDAP Panel, 2005), and on the safety and efficacy of several zinc compounds: zinc chelate of hydroxy analogue of methionine (Mintrex®Zn) (EFSA FEEDAP Panel, 2008a; EFSA FEEDAP Panel, 2009a,b), of tetra-basic zinc chloride (EFSA FEEDAP Panel, 2012a), of methionine zinc (EFSA FEEDAP Panel, 2013a) and of zinc chelate of L-lysinate-HCl (EFSA FEEDAP Panel, 2015a). In the frame of re-evaluation, EFSA has delivered six opinions on seven zinc-based additives – including zinc acetate, dihydrate; zinc chloride, anhydrous; zinc oxide; zinc sulfate, heptahydrate; zinc sulfate, monohydrate; zinc chelate of amino acids, hydrate; zinc chelate of glycine, hydrate (EFSA FEEDAP Panel, 2012b–e, 2013b, 2015b).

EFSA has also issued an opinion on the potential reduction of the currently authorised maximum zinc content in complete feed. The newly proposed maximum contents were: 150 mg Zn/kg complete feed for piglets, sows, rabbits, salmonids, cats and dogs; 120 mg Zn/kg complete feed for turkeys for fattening; 100 mg Zn/kg complete feed for all other species and categories (EFSA FEEDAP Panel, 2014).

'Zinc chelate of methionine sulfate' is not authorised as a source of zinc in animal nutrition, being this one the first application in the European Union (EU) for such use as compound of trace elements. Several zinc compounds are authorised to be used as nutritional feed additives (trace elements) in the EU: 'Zinc lactate, trihydrate', 'Zinc carbonate', 'Zinc chloride, monohydrate' (Commission Regulation (EC) No 1334/2003[4] (modified by Commission Regulation (EC) No 1980/2005[5]), 'Zinc chelate of hydroxy analogue of methionine' (Commission Regulation (EC) No 888/2009[6]; Commission Regulation (EU) No 335/2010[7]), 'Zinc chloride hydroxide monohydrate' (Commission Implementing Regulation (EU) No 991/2012[8]) and 'Zinc chelate of methionine (1:2)' (Commission Implementing Regulation (EU)

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] Norel, S.A. C/Jesús Aprendiz 19, 1° A y B. 28007 Madrid. Spain.
[3] The name of the additive is: Zinc chelate of methionine sulfate (see Section 3.1).
[4] Commission Regulation (EC) No 1334/2003 of 25 July 2003 amending the conditions for authorisation of a number of additives in feedingstuffs belonging to the group of trace elements. OJ L 187, 26.7.2003, p. 11.
[5] Commission Regulation (EC) No 1980/2005 of 5 December 2005 amending the conditions for authorisation of a feed additive belonging to the group of trace elements and of a feed additive belonging to the group of binders and anti-caking agents. OJ L 318, 6.12.2005, p. 3.
[6] Commission Regulation (EC) No 888/2009 of 25 September 2009 concerning the authorisation of Zinc chelate of hydroxy analogue of methionine as a feed additive for chickens for fattening. OJ L 254, 26.9.2009, p. 71.
[7] Commission Regulation (EU) No 335/2010 of 22 April 2010 concerning the authorisation of zinc chelate of hydroxy analogue of methionine as a feed additive for all animal species. OJ L 102, 23.4.2010, p. 22.
[8] Commission Implementing Regulation (EU) No 991/2012 of 25 October 2012 concerning the authorisation of zinc chloride hydroxide monohydrate as feed additive for all animal species. OJ L 297, 26.11.2012, p. 18.

No 636/2013[9]). Following the re-evaluation of feed additives, the authorisation of various zinc compounds as nutritional additives has been regranted: 'Zinc acetate dehydrate', 'Zinc chloride anhydrous', 'Zinc oxide', 'Zinc sulfate heptahydrate', 'Zinc sulfate monohydrate', 'Zinc chelate of amino acids hydrate', 'Zinc chelate of protein hydrolysates', and 'Zinc chelate of glycine hydrate (solid and liquid)' (Commission Implementing Regulation (EU) 2016/1095[10]).

Zinc methionine complex is approved as a feed ingredient for use in the United States (AAFCO, 2014).

An opinion for the use of zinc mono L-methionine sulfate (CAS No 56329-42-1) in food was adopted by the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS Panel) (EFSA ANS Panel, 2009); the compound was subsequently authorised by Commission Regulation (EC) No 1170/2009 as mineral which may be used in the manufacture of food supplements.

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[11] in support of the authorisation request for the use of zinc chelate of methionine sulfate 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[12] and the applicable EFSA guidance documents.

The EFSA Panel on Additives and Products or Substances used in Animal Feed (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 and other scientific reports 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 zinc chelate of methionine in animal feed. The Executive Summary of the EURL report can be found in Annex A.[13]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of zinc chelate of methionine sulfate is in line with the principles laid down in Regulation (EC) No 429/2008[12] and the relevant guidance documents: Guidance on nutritional additives (EFSA FEEDAP Panel, 2012f), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012g), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012h), Technical Guidance for assessing the safety of feed additives for the environment (EFSA FEEDAP Panel, 2008b) and Guidance for the preparation of dossiers for additives already authorised for use in food (EFSA FEEDAP Panel, 2012i).

3. Assessment

The additive under application, 'zinc chelate of methionine sulfate', is zinc chelated with methionine in a molar ratio 1:1. It is intended to supply zinc as a nutritional additive to all animal species/categories.

A formulated additive is intended to be manufactured and marketed as BIOMET®Zn 15%, 10% and 2%, with a zinc concentration of 15%, 10% and 2%, respectively; calcium carbonate is used as an excipient in the formulation.

[9] Commission Implementing Regulation (EU) No 636/2013 concerning the authorisation of zinc chelate of methionine (1:2) as a feed additive for all animal species. OJ L 183, 2.7.2013, p. 3.

[10] Commission Implementing Regulation (EU) 2016/1095 of 6 July 2016 concerning the authorisation of Zinc acetate dihydrate, Zinc chloride anhydrous, Zinc oxide, Zinc sulfate heptahydrate, Zinc sulfate monohydrate, Zinc chelate of amino acids hydrate, Zinc chelate of protein hydrolysates, Zinc chelate of glycine hydrate (solid) and Zinc chelate of glycine hydrate (liquid) as feed additives for all animal species and amending Regulations (EC) No 1334/2003, (EC) No 479/2006, (EU) No 335/2010 and Implementing Regulations (EU) No 991/2012 and (EU) No 636/2013. OJ L 182, 7.7.2016, p. 7.

[11] FEED dossier reference: FAD-2015-0025.

[12] 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.

[13] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finrep-fad-2015-0025-zinc-methionine.pdf

3.1. Characterisation

'Zinc chelate of methionine sulfate' is described in the technical dossier with the Chemical Abstracts Service (CAS) No 56329-42-1.[14] Its corresponding International Union of Pure and Applied Chemistry (IUPAC) name is 'Zinc, 2-amino-4 methylsulfanylbutanoic acid, sulfate'. Its chemical formula is $C_5H_{11}NO_6S_2Zn$, and it has a molecular weight of 310.66 Da. The theoretical zinc content is 21.05%. The structural formula of the compound, corresponding to the IUPAC name, is shown in Figure 1.[15]

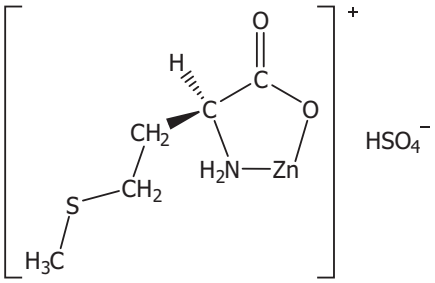

Figure 1: Structural formula of 'Zinc, 2-amino-4 methylsulfanylbutanoic acid, sulfate'

The additive is a white solid powder. Its solubility in water is 4.2 g/100 mL water.[16] Bulk density is 500 kg/m^3.[17]

Analytical data of five batches of the compound showed an average zinc content of 19.5% (range: 19.1–20.8), a methionine content of 42.8% (range: 41.2–46.0) and water of 2.5% (range: 1.6–3.1).[18] The balance of the composition (range: 31.7–37.5) is assumed to be mostly hydrogen sulfate, as its theoretical content in the compound is 31.2%.

Impurities were tested in three batches of the compound.[19] The results showed contents of arsenic ranging from 0.115 to 0.252 mg/kg, of cadmium from 0.230 to 0.825 mg/kg and of lead from 0.212 to 0.903 mg/kg additive. Mercury concentrations were below the detection limit (< 0.025 mg/kg) in all samples. Concentrations of dioxin and dioxin-like PCBs were reported for the same batches: Dioxins = 0.029–0.032 ng WHO-PCDD/F-TEQ per kg; sum dioxins plus dioxin-like PCBs = 0.040–0.045 ng WHO-PCDD/F-PCB-TEQ per kg. All these values comply with those specified in Directive 2002/32/EC for compounds of trace elements or, if not mentioned, do not pose concerns.

The particle size distribution was analysed by laser diffraction in three batches of the compound.[20] Results showed that, on average, 21.7%, 64.8% and 88.6% of the particles had a diameter < 10, < 50 and < 100 μm, respectively. The dusting potential of the same three batches measured by the Stauber–Heubach method ranged from 2.16 to 2.77 g/m^3.[21]

3.1.1. Production process

The manufacturing process is fully described in the technical dossier. Basically, it is a chelation reaction between zinc sulfate heptahydrate and DL-methionine. Zinc chelate of methionine hydrate is manufactured by mixing commercial DL-methionine with inorganic zinc sulfate heptahydrate under specific heat and moisture conditions in a reactor/dryer.

The effectiveness of the chelation reaction is monitored by the disappearance of free methionine in the final reaction product by means of infrared absorption spectrometry, in which the absence of the band at 2,100/cm corresponding to free methionine was observed.[22]

For the manufacture of the formulated additive, the dry zinc chelate of methionine sulfate is sieved to a standard particle size and then blended with feed grade calcium carbonate (carrier).

[14] The correct denomination of the additive is 'Zinc chelate of methionine sulfate' (not 'Zinc chelate of methionine hydrate' (name proposed by the applicant)), which corresponds to the CAS number provided.
[15] The FEEDAP Panel has drawn a recommendation concerning the modification of the name of the compound.
[16] Technical Dossier/Supplementary Information (September 2016)/Annex 4.1.
[17] Technical Dossier/Supplementary Information (September 2016).
[18] Technical Dossier/Supplementary Information (September 2016)/Annex 1.1.
[19] Technical Dossier/Supplementary Information (September 2016)/Annex 2.1.
[20] Technical Dossier/Supplementary Information (September 2016)/Annexes 3.1 and 3.2.
[21] Technical Dossier/Supplementary Information (September 2016)/Annex 3.3.
[22] Technical Dossier/Section II/Annex II.1.1.

3.1.2. Stability and homogeneity

For compounds of trace elements (including chelates), stability studies are generally not required. If specific effects are indicated or claimed for a particular form of the trace element (e.g. organometallic compounds, nanoparticles), the stability of that specific form of the additive should be followed. The applicant proposes a shelf-life of 18 months when stored in a dry place (formulated additive at 2% zinc concentration).[23] The applicant indicated that the additive should be stored in a dry place.[24]

The applicant was requested to submit data for demonstration of the stability of the additive but provided data on stability of one of the formulations of the additive.[25] It was evaluated by measuring the zinc and methionine content of three batches of BIOMET®15% after ca 2 years storage (19–21 months) at room temperature (18–20°C). The differences between the initial and the final content of zinc and methionine were very small, demonstrating that the zinc and methionine levels in the additive are stable during the shelf-life indicated by the applicant (18 months).

A study to support homogenous distribution of the additive in feed was provided.[26] Three different batches of dog feed supplemented with BIOMETZn®15% up to an intended total zinc feed concentration of 150, 200 and 250 mg/kg were analysed 10 times (no data on the ingredient composition and background zinc concentration of the dog feed were provided). The coefficients of variation were 7.5%, 4.5% and 5.3% for the diets containing 160, 215 and 251 mg Zn/kg feed respectively.

3.1.3. Physicochemical incompatibilities in feed

No incompatibilities with feed components, carriers, other approved additives or medical products are to be expected, other than those already widely recognised for zinc in animal nutrition (see EFSA FEEDAP Panel, 2014).

3.1.4. Conditions of use

The zinc compound under application, 'zinc chelate of methionine sulfate', is intended to supply zinc in final feed for all animal species/categories up to a maximum total content in complete feedingstuffs of 200 mg Zn/kg for dogs and cats, 180 mg Zn/kg for salmonids and milk replacers for calves, 150 mg Zn/kg for piglets, sows, rabbits and all fish species other than salmonids, and 120 mg Zn/kg for other species and categories. These values are in agreement with the maximum total content of zinc in feed set in Commission implementing Regulation (EU) 2016/1095[10].

Zinc chelate of methionine sulfate is intended to be mixed into feedingstuffs either directly to the feed or via a premixture.

3.2. Safety

3.2.1. Toxicological studies

The additive 'zinc chelate of methionine sulfate' is very likely dissociated, after absorption, into its two main components, zinc and methionine.

Methionine from the additive is completely metabolised. Adverse effects of excess supplemented methionine in farm animals are related to the induction of amino acid imbalance (EFSA FEEDAP Panel, 2012j). Nevertheless, based on maximum authorised levels of zinc in feed for different species, the additive under assessment will provide a methionine concentration in feeds of 300–400 mg/kg feed; this concentration is markedly lower than that usually provided by methionine as feed additive (e.g. 2,500 mg supplemental methionine/kg feed for chickens for fattening).

The toxicological properties of zinc, including methionine-zinc, have been discussed in detail by the Scientific Committee on Food (SCF) (EC, 2003b). The toxicology of zinc has been recently reviewed by Sandstead (2015) and also extensively described in EFSA FEEDAP opinions (e.g. EFSA FEEDAP Panel 2012b–e, 2015b); no substantial differences between inorganic and organic zinc compounds are expected concerning zinc toxicity. Briefly it can be summarised that:

- depressed copper uptake with associated copper deficiency is the most sensitive and well-characterised effect of chronic excess of zinc intake in humans and animals (Maret and

[23] Technical Dossier/Section II/Annex II.4. (Certificate of analysis of Biomet®Zn 2%).
[24] Technical Dossier/Section II/Annex II.10.
[25] Technical Dossier/Supplementary Information (September 2016)/Annex 5.1.
[26] Technical Dossier/Supplementary Information (September 2016)/Annex 6.1.

Sandstead, 2006; see also Van Paemel et al., 2010). Accordingly, a tolerable upper intake level (UL) for zinc of 25 mg/day in adults is derived from a human no observed adverse effect level (NOAEL) of 50 mg/day in adults for altered copper status and an uncertainty factor of 2 to allow for observed variability within the general population (EC, 2003b);

- whereas some positive results (chromosome aberrations) were observed in genotoxicity tests with high doses of zinc compounds, the weight of evidence from the *in vitro* and *in vivo* genotoxicity tests supports the conclusion that zinc has no biologically relevant genotoxic activity (WHO, 2001; ATSDR, 2005).

3.2.2. Safety for the target species

Where application for all animal species is made for a nutritional additive, tolerance data may be limited to one species. To investigate the safety for chickens for fattening, a feeding trial, conducted in two consecutive runs, with a scheduled number of a total of 300 one-day-old Cobb chickens (mixed gender) was carried out.[27] The chickens were distributed to five experimental treatments with six pens of ten chickens per treatment (three pens per treatment and run). However, only a reduced number of replicates (pens) per treatment was reported, corresponding to a total of 260 day-old chickens. All chickens were fed *ad libitum* with mash feed (starter/grower) based on maize (31.9/33.4%), barley (20.0/23.3%), soybean meal (33.5/36%) and lard (6.6/7.3%) during the starting (days 0–21) and growing (21–35 days) period.

The control animals were given the unsupplemented diets which contained 31 and 30 and mg Zn/kg starter and grower feed, respectively. The experimental groups received diets with 150 and 450 mg supplemental Zn/kg feed from either zinc sulfate monohydrate or zinc chelate of methionine sulfate. The intended zinc concentrations in the diets were confirmed by analysis. Mortality, feed consumption and body weight were measured on days 21 and 35. At the end of the study, eight randomly selected birds per treatment (four males and four females, identified with leg rings and allocated to different pens) were subjected to blood sampling for analysis of blood chemistry[28] and haematology[29] parameters. At the same time, chickens were slaughtered and samples of skin, fat pad, bone, breast meat, liver, kidneys and pancreas were collected from one bird per pen for zinc analysis.

The statistical unit was the pen for animal performance and the individual bird for blood and tissue deposition. All data were subjected to analysis of variance (ANOVA) followed by Tukey test for group differences.

The study showed some weaknesses: (i) the study was conducted in two consecutive runs, (ii) the reason for the differences in the number of intended and assessed replicates was not given, (iii) the number of animals taken for blood sampling was not equally distributed among the two runs (three males and two females in the first run, one male and two females in the second run), (iv) the contribution of methionine (up to 935 mg/kg feed) from zinc chelate of methionine sulfate was not considered in the diet formulation (e.g. by lower supplementation of DL-methionine).

Due to these limitations, this study cannot be used for the assessment of safety for target animals. Therefore, the FEEDAP Panel cannot conclude on the safety of zinc chelate of methionine sulfate for the target species.

3.2.3. Safety for the consumer

The FEEDAP Panel has summarised, in several opinions (EFSA FEEDAP Panel, 2012b–e, 2014, 2015b) the zinc intake of the European population, as derived from various sources (EC, 2003b; Mensink et al., 2007; Flynn et al., 2009; Rubio et al., 2009; Turconi et al., 2009) and the possible contribution of zinc supplementation of animal feeds. In all consumer groups, tissues and products of animal origin contributed to about 40–50% of total zinc intake. In its previously adopted opinion on the potential reduction of the currently authorised maximum zinc content in complete feed, the FEEDAP Panel concluded that the newly proposed reduced maximum zinc levels would not, essentially, influence consumer exposure (EFSA FEEDAP Panel, 2014). There is no indication that the current intake levels in the European population, which already include food commodities from zinc-supplemented animals, may be higher than the UL (25 mg Zn/day in adults) (EFSA NDA Panel, 2014).

[27] Technical Dossier/Section III/Annex III.1; Technical Dossier/Supplementary Information (September 2016); Technical Dossier/Supplementary Information (February 2017).
[28] Urea, creatinine, total proteins, albumin, glucose, bilirubin, sodium, calcium, phosphorous, glutamate pyruvate transaminase, glutamic-oxaloacetic transaminase, alkaline phosphatase, lactate dehydrogenase, ceramide galactosyltransferase.
[29] Erythrocytes, leucocytes, heterophils, lymphocytes, monocytes, eosinophils, haematocrit, thrombocytes.

The data summarised in the SCAN opinion (EC, 2003a,b) indicate that the use of zinc chelate of methionine sulfate as that of other compounds of zinc would not significantly modify the zinc content of edible tissues; numerical differences are small and may also be related to presence of other dietary ingredients that interfere with absorption and/or utilisation of zinc. In summary, the exposure of consumers to zinc form tissues and products of animal origin would also not essentially be modified by the use of the zinc compound under assessment in animal nutrition.

Methionine is incorporated in the protein of tissues and products, showing a constant amino acid pattern. Consequently, the use of the additive reported here will not result in an increased content of sulfur containing amino acids in tissues and products. Doses exceeding the requirement will be metabolised and excreted.

3.2.3.1. Conclusions on safety for the consumer

No concerns for consumer safety are expected from the use of the zinc chelate of methionine sulfate in animal nutrition when used up to the maximum EU authorised zinc levels in feed.

3.2.4. Safety for the user

No specific studies have been submitted for the additive under assessment.

The hazards for skin and mucosae posed by zinc compounds in occupational settings are well recognised and documented. Many zinc compounds are skin and eye irritants (EFSA FEEDAP Panel, 2015b); the FEEDAP Panel considers that read-across is appropriate for zinc chelate of methionine sulfate; thus, the additive should be considered as a skin and eye irritant. Zinc (metal) may cause sensitisation by skin contact; also considering its methionine component, the additive should be considered as a skin sensitiser.

Zinc is considered hazardous by inhalation; the inhalation toxicity of the specific zinc compounds is closely related to the respirable fraction in the dust. Zinc chelate of methionine sulfate has high dusting potential (up to 2.77 g/m^3) and a respirable fraction up to 22%; assuming that the zinc content of the dust is the same as the additive (approximately 20%), this leads to 2.4, 12 and 18.3 mg/m^3 of respirable zinc from the 2%, 10% and 15% formulations, respectively. The American Conference of Governmental Industrial Hygienists (ACGIH) proposed a threshold limit value (TLV) for zinc oxide of 2 mg/m^3 based on metal fume fever as the critical effect (ACGIH, 2003). The concentration of zinc in the dust from the additive zinc chelate of methionine sulfate therefore is slightly above the TLV for the 2% formulation but exceeds the TLV by one order of magnitude for the 10% and 15% formulations. The FEEDAP Panel recognises that occupational exposure limits such as the TLV are elaborated for the continuous exposure of factory workers; their use as such is likely to be overly conservative considering the user exposure to feed additives, consequently an estimated exposure slightly exceeding the TLV is not considered to represent a concern. Nevertheless, the FEEDAP Panel considers that an exceedance of the TLV by one magnitude order would indicate a risk to users. Therefore the 10% and 15% additive formulations are considered to pose a risk by inhalation to the users.

3.2.4.1. Conclusions on safety for the user

Zinc chelate of methionine sulfate should be considered as a skin and eye irritant, and as a skin sensitiser. The additive itself and when used in higher concentrated formulations (e.g. 10% or 15%) is considered to pose a risk to users by inhalation.

3.2.5. Safety for the environment

The additive under assessment, zinc chelate of methionine sulfate, is intended to be a substitute for other authorised zinc additives and will not further increase the environmental burden of zinc. Therefore, the FEEDAP Panel considers that the use of the additive in animal nutrition would not pose an additional risk for the environment.

3.3. Efficacy

To support efficacy, the applicant provided a literature review, performed a balance trial in chickens for fattening and, proposed to use the data from the tolerance study in chickens for fattening. However, due to the weaknesses identified, the latter study could not be considered.

3.3.1. Literature review efficacy trials

The applicant submitted some scientific papers to support efficacy of zinc methionine chelate. Only those reporting *in vivo* studies conducted within the EU currently authorised maximum zinc levels (see Section 3.1.4) were selected as efficacy trials by the FEEDAP Panel for the purpose of the current assessment. Furthermore, the Panel notes that there are different zinc methionine chelates in use: in the relevant literature mainly the forms with the molar ratio of zinc to methionine 1:2 and that with the ratio of 1:1 are reported; the latter one corresponds to the compound under assessment, and it is often called as 'zinc methionine complex' and referred as such in this section. Only those studies in which the authors used the same test item as the zinc chelate under assessment, were further considered. The relevant studies meeting the said criteria are summarised below.

Spears (1989) conducted four trials (three in lambs and one with heifers)[30] to evaluate zinc methionine complex as a zinc source for ruminants in comparison with zinc oxide. In one experiment, the author added 5 mg Zn from each of the zinc sources to 1 kg lamb diet (background content 2.8 mg Zn/kg) fed for 42 days (depletion phase); after 7 days of the end of the depletion phase, 15 mg Zn/kg feed were given in the balance part of the study. No differences in zinc availability derived from plasma zinc and plasma alkaline phosphatase activity were detected between both zinc sources. In a second experiment, 20 mg/kg of each of the zinc sources were added to a diet (mainly chopped orchard grass) containing 30 mg Zn/kg: zinc absorption and retention by lambs were similar for zinc oxide and zinc methionine complex. In another experiment, 36 Hereford x Simmental heifers were used to evaluate the effects of 25 mg Zn from each of the sources per kg diet (zinc background: 23 mg/kg); the trial lasted 126 days. No significant effects were observed in growth performance of both zinc sources; plasma zinc and alkaline phosphatase contents were not affected by zinc source.

Wedekind et al. (1992) tested three different basal diets (based on (i) amino acid mix and corn starch/oil, (ii) soybean isolate, dextrose and corn oil and (iii) corn-soybean meal and corn oil, containing a zinc background of 1, 13 and 45 mg/kg diet, respectively) and measured the bioavailability of zinc from zinc oxide, zinc sulfate and zinc methionine complex in five trials with chickens for fattening during 14 days (from 8 to 22 days post-hatching). The zinc supplementation varied across the trials, but in all, except in trial 3 the supplemental doses were within the limits allowed in the EU (up to 50 mg supplemental Zn/kg diet). Feed intake, weight gain of chicken and zinc concentration in tibia, plasma, pancreas, liver and kidney were measured. The authors estimated the zinc bioavailability from zinc methionine complex relative to zinc sulfate as > 100% under all experimental diets.

Wedekind et al. (1994) conducted one experiment with pigs (32 pigs per treatment; 20 kg initial body weight) to compare the effect of zinc sulfate monohydrate supplementation (0, 7.5 and 15 mg/kg) with zinc oxide (15 mg Zn/kg), zinc methionine complex and zinc lysine (both 7.5 mg Zn/kg). The basal zinc content of the diet amounted to 32 and 27 mg/kg for the growing and finishing periods respectively. Feed intake and daily weight gain were not significantly influenced during growing (20–55 kg) and finishing (> 55 kg body weight) period. The authors calculated values of zinc bioavailability based on the zinc content of plasma and bone (metacarpals; Coccygeal vertebrae) and described an overall bioavailability ranking: zinc sulfate > zinc methionine complex > zinc oxide > zinc lysine.[31]

The FEEDAP Panel identified further studies. From the review of De Groote et al. (2002) and the study of Van Heugten et al. (2003) in poultry, pigs and ruminants, it was confirmed that zinc methionine complex is at least as bioavailable as zinc sulfate or zinc oxide.

3.3.2. Bioavailability study

The applicant submitted a short-term efficacy study conducted with chickens for fattening.[32,33] A total of 96 one-day-old Cobb 300 chickens (male and female) were distributed to two experimental treatments: (a) positive control with zinc sulfate and (b) experimental group with zinc chelate of methionine sulfate. The chickens were allocated into eight pens per treatment with six chickens per pen. The basal diet (zinc background content: 28 mg/kg) consisted mainly of maize, wheat, barley and soybean meal and was given as mash feed. Zinc from each of the test items was added at 220 mg/kg feed. Feed intake and faeces amounts were recorded from day 17 to 20 of age and zinc concentrations

[30] The Experiment 3 in lambs was not considered by the FEEDAP Panel because of the high zinc supplementation (300 mg/kg).
[31] E.g. based on zinc in metacarpal bone the relative bioavailability compared to zinc sulfate was estimated to be: 60.4, 66.7 and 37.5 for zinc methionine complex, zinc oxide and zinc lysine, respectively.
[32] Technical Dossier/Section IV/Annex IV.1.
[33] Technical Dossier/Supplementary Information (February 2017)/Annex IV_1.

were analysed. The retention of the zinc sources was 14.7% and 14.8% for zinc sulfate and zinc chelate, respectively. The FEEDAP Panel notes that the zinc content of the feed in this study exceeded the maximum allowed in the EU for chickens feed; notwithstanding this caveat, the data allow the conclusion that both sources are equally bioavailable.

3.3.3. Conclusions on the efficacy

Based on literature studies and a specific study conducted with the additive under assessment, zinc chelate of methionine sulfate is an available source of zinc for all animal species.

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[34] and Good Manufacturing Practice.

4. Conclusions

Owing to the limitations of the tolerance study, it could not be used for the assessment of safety for target animals; therefore, the FEEDAP Panel cannot conclude on the safety of zinc chelate of methionine sulfate for the target species.

No concerns for consumer safety are expected from the use of the zinc chelate of methionine sulfate in animal nutrition when used up to the maximum EU authorised zinc levels in feed.

Zinc chelate of methionine sulfate should be considered as a skin and eye irritant, and as a skin sensitiser; it is considered to pose a risk by inhalation to the users.

The additive under assessment, zinc chelate of methionine sulfate, is intended to be a substitute for other authorised zinc additives and will not further increase the environmental burden of zinc; therefore, the FEEDAP Panel considers that the use of the additive in animal nutrition would not pose an additional risk for the environment.

Based on literature studies and a specific study conducted with the additive under assessment, zinc chelate of methionine sulfate is an available source of zinc for all animal species.

Documentation provided to EFSA

1) Zinc chelate of methionine hydrate (BIOMET Zn) for all animal species. July 2015. Submitted by Norel S.A.
2) Zinc chelate of methionine hydrate (BIOMET Zn) for all animal species. Supplementary information. September 2016. Submitted by Norel S.A.
3) Zinc chelate of methionine hydrate (BIOMET Zn) for all animal species. Supplementary information. February 2017. Submitted by Norel S.A.
4) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for Zinc Chelate of Methionine.
5) Comments from Member States.

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Abbreviations

ACGIH	American Conference of Governmental Industrial Hygienists
ANOVA	analysis of variance
ANS	EFSA Panel on Food Additives and Nutrient Sources added to Food
CAS	Chemical Abstracts Service
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
ICP-AES	inductively coupled plasma atomic emission spectroscopy
IUPAC	International Union of Pure and Applied Chemistry
NOAEL	No Observed Adverse Effect Level
PCB	polychlorinated biphenyl

PCDD/F	polychlorinated dibenzo-p-dioxins and dibenzofurans
SCAN	Scientific Committee on Animal Nutrition
SCF	Scientific Committee on Food
TLV	threshold limit value
UL	tolerable upper intake level

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Zinc Chelate of Methionine

In the current application authorisation is sought under article 4(1) for *Zinc Chelate of Methionine* 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* (*Zinc Chelate of Methionine*) consists of equimolar amounts of zinc and methionine, and is to be marketed as three powder formulations containing minimum contents of 2, 10, and 15% *zinc*; and 4.6, 23 and 36% *methionine*, respectively. The *feed additive* is intended to be incorporated directly into *feedingstuffs* or through *premixtures*. The Applicant suggested maximum levels of *total zinc* in complete *feedingstuffs* complying with the limits set in Regulation (EC) No 1334/2003, ranging from 150 to 250 mg/kg – depending of the animal species/category.

For the determination of *total zinc* in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted the internationally recognised ring-trial validated method EN 15510 based on inductively coupled plasma atomic emission spectroscopy (ICP-AES). Two additional methods were previously evaluated and recommended by the EURL in the frame of the Zinc group dossier: the ring-trial validated EN 15621 method based on ICP-AES after pressure digestion and the Community method based on atomic absorption spectroscopy. The Community method was further ring-trial validated by the UK Food Standards Agency (FSA). Based on the performance characteristics available, the EURL recommends for official control the two CEN methods (EN 15510 or EN 15621) for the quantification of *total zinc* in the *feed additive*, *premixtures* and *feedingstuffs*, together with the Community method (Com Reg (EC) No 152/2009 – Annex IV-C) for the quantification of *total zinc* in feedingstuffs.

For the quantification of *methionine* content in the *feed additive* the Applicant submitted the Official AOAC 999.13 method, based on ion-exchange chromatography coupled with postcolumn derivatisation and colourimetric or fluorescence detection. Two additional equivalent to the AOAC ring-trial validated methods were previously evaluated and recommended by the EURL in the frame of previous dossiers (FAD-2015-0002, FAD-2010-0023 and FAD-2010-0254): EN ISO 17180 and VDLUFA 4.11.6), based on ion-exchange chromatography coupled with post-column derivatisation and colourimetric or fluorescence detection. Based on acceptable performance characteristics available, the EURL recommends for official control the two ring-trial validated methods (EN ISO 17180 and VDLUFA 4.11.) to quantify *methionine* content in the *feed additive*.

For the quantification of *total methionine* in *feedingstuffs* the EURL already evaluated and recommended for official control - in the frame of the Methionine group dossier (FAD-2010-0023) - the ring-trial validated Community method. This method applies for the determination EURL Evaluation Report "Zinc Chelate of Methionine" of free (synthetic and natural) and of total (peptide-bound and free) amino acids, using an amino acid analyzer or High Performance Liquid Chromatography (HPLC) equipment. The method does not distinguish between the salts and the amino acid enantiomers.

This method was further ring-trial validated in protein concentrate, premixture and feed, resulting in the EN ISO 13903 method. Since the validation range of the method for *methionine* concentration covers the *methionine* content in *premixtures* and the formulation with lowest *methionine* content of 4.6%, in addition, the EURL recommends for official control this method to quantify *methionine* content in the *feed additive*.

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.