European Food Safety Authority

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SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of zinc compounds (E6) as feed additives for all animal species (zinc acetate, dihydrate; zinc chloride, anhydrous; zinc oxide; zinc sulphate, heptahydrate; zinc sulphate, monohydrate; zinc chelate of amino acids, hydrate; zinc chelate of glycine, hydrate), based on a dossier submitted by FEFANA asbl[1]

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) has assessed seven zinc compounds in the current application: zinc acetate, dihydrate; zinc chloride, anhydrous; zinc oxide; zinc sulphate, heptahydrate; zinc sulphate, monohydrate; zinc chelate of amino acids, hydrate; zinc chelate of glycine, hydrate. These compounds are safe sources of zinc for all animal species/categories when used up to maximum authorised zinc levels in feed; the simultaneous use of both feed and water supplemented with zinc should be avoided. No concerns for consumer safety are expected from the use of the zinc compounds under application when used up to the maximum authorised levels in feed. Zinc acetate, zinc sulphate (heptahydrate and monohydrate), zinc chelate of amino acids, hydrate, and zinc chelate of glycine, hydrate, are irritant to the skin, eyes and mucosae. Zinc sulphate may cause skin sensitisation. Zinc chloride, anhydrous, is extremely corrosive to skin, eyes and mucosae. Zinc chelate of amino acids, hydrate, may induce sensitisation by inhalation and should be considered a skin sensitiser. Zinc oxide is not irritant to skin or eyes or skin sensitiser. The Panel considers that all the additives under application should be treated as hazardous by inhalation. The use of the zinc compounds under assessment does not pose an immediate concern for the agricultural soil compartment. However, there is a potential environmental concern related to drainage and the runoff of zinc to surface water, with acidic sandy soils being the most vulnerable; for a final conclusion, further refinements to the assessment of zinc-based additives in livestock need to be considered, and additional data are required. The adoption of the newly proposed maximum zinc contents in feeds would greatly reduce the risk to the environment from zinc-containing additives. The zinc compounds under assessment are efficacious in meeting animal requirements.

KEY WORDS

nutritional additive, compounds of trace elements, zinc, zinc compounds, safety, environment, efficacy

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[1] On request from the European Commission, Question No EFSA-Q-2011-00845, adopted on 12 March 2015.

[2] Panel members: Gabriele Aquilina, Vasileios Bampidis, Maria De Lourdes Bastos, Lucio Guido Costa, Gerhard Flachowsky, Mikolaj Antoni Gralak, Christer Hogstrand, Lubomir Leng, Secundino López-Puente, Giovanna Martelli, Baltasar Mayo, Fernando Ramos, Derek Renshaw, Guido Rychen, Maria Saarela, Kristen Sejrsen, Patrick Van Beelen, Robert John Wallace and Johannes Westendorf. Correspondence: FEEDAP@efsa.europa.eu

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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 the safety and efficacy of the following zinc compounds as feed additives for all animal species: zinc acetate, dihydrate; zinc chloride, anhydrous; zinc oxide; zinc sulphate, heptahydrate; zinc sulphate, monohydrate; zinc chelate of amino acids, hydrate; and zinc chelate of glycine, hydrate.

The FEEDAP Panel concludes that all the zinc compounds under application are safe sources of zinc for all animal species/categories when used up to maximum European Union-authorised zinc levels in complete feed. The simultaneous use of both feed and water supplemented with zinc should be avoided.

No concerns for consumer safety are expected from the use of the zinc compounds under application in animal nutrition when used up to maximum EU-authorised levels in feed.

Zinc acetate, zinc sulphate (heptahydrate and monohydrate), zinc chelate of amino acids, hydrate, and zinc chelate of glycine, hydrate, are irritant to the skin, eyes and mucosae. Zinc sulphate may cause skin sensitisation. Zinc chloride, anhydrous, is extremely corrosive to skin, eyes and mucosae. Zinc chelate of amino acids, hydrate, may induce sensitisation by inhalation and should be considered a skin sensitiser. Zinc oxide is not considered an irritant for skin or eyes or a skin sensitiser. The FEEDAP Panel considers that all the additives under application should be treated as hazardous by inhalation.

The use of the zinc compounds under the current application as feed additives does not pose an immediate concern for the agricultural soil compartment. However, there is a potential environmental concern related to drainage and the runoff of zinc to surface water; the most vulnerable to these processes are acidic sandy soils. In order to draw a final conclusion, some further refinements to the assessment of zinc-based feed additives in livestock need to be considered, for which additional data are required. With the adoption of the newly proposed maximum zinc contents in animal feeds, the risk to the environment, from the use of zinc-containing feed additives, would be greatly reduced.

The zinc compounds under the current application are efficacious in meeting animal zinc requirements.

The FEEDAP Panel made some recommendations concerning (i) the characterisation of zinc chloride, anhydrous, and of zinc chelate of amino acids, hydrate; (ii) the conditions of use of zinc chloride, anhydrous; (iii) measures to avoid risks to users/workers; (iv) the use of zinc-based additives in water for drinking, which is seen as critical to ensure compliance with the legally established maximum supply of zinc to animals.

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BACKGROUND

Regulation (EC) No 1831/2003[4] establishes the rules governing the Community authorisation of additives for use in animal nutrition. 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. 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 time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from TREAC EEIG (Trace Elements Authorisation Consortium European Economic Interest Grouping)[5] for (i) authorisation of a new additive, (ii) authorisation of a new use and (iii) re-evaluation of authorisation, of seven zinc-containing additives (zinc acetate, dihydrate; zinc chloride, anhydrous; zinc oxide; zinc sulphate, heptahydrate; zinc sulphate, monohydrate; zinc chelate of amino acids, hydrate; zinc chelate of glycine, hydrate) when used as feed additives 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) under Article 4(1) (authorisation of a feed additive or new use of a feed additive) and under Article 10(2) (re-evaluation of an authorised feed additive). EFSA received directly from the applicant the technical dossiers in support of this application.[6] According to Article 8 of that Regulation, 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. The particulars and documents in support of the application were considered valid by EFSA as of 2 August 2011.

The additives 'Zinc acetate, dihydrate', 'Zinc oxide', 'Zinc sulphate, heptahydrate', 'Zinc sulphate, monohydrate', 'Zinc chelate of amino acids, hydrate' and 'Zinc chelate of glycine, hydrate' had been authorised in the EU under the element Zinc-Zn (E6) for all animal species 'Without a time limit' (Commission Regulation (EC) No 1334/2003[7] and Commission Regulation (EC) No 479/2006)[8] and amendments. Following the provisions of Article 10(1) of Regulation (EC) No 1831/2003 the compounds were included in the EU Register of Feed Additives under the category 'Nutritional additives' and the functional group 'Compounds of trace elements'.[9] The additive 'Zinc chloride, anhydrous' has not been previously authorised in the EU.

The Scientific Committee on Animal Nutrition (SCAN) issued an opinion on the use of zinc in feedingstuffs (EC, 2003a). EFSA issued opinions on the safety of the chelated forms of iron, copper, manganese and zinc with synthetic feed grade glycine (EFSA, 2005), on the safety and efficacy of a zinc chelate of hydroxy analogue of zinc (Mintrex®Zn) (EFSA, 2008a, 2009a, 2009b), of tetra-basic zinc chloride (EFSA, 2012a) and of methionine-zinc (EFSA FEEDAP Panel, 2013a) as feed additives (compounds of trace elements). In the frame of re-evaluation, EFSA has delivered five opinions on

[4] 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.

[5] On 13/03/2013, EFSA was informed by the applicant that TREAC EEIG was liquidated on 19/12/2012 and their rights as applicant were transferred to FEFANA asbl (EU Association of Specialty Feed Ingredients and their Mixtures), including the 14 companies referred to in the text as (d1) to (d14). Avenue Louise 130A-Box 1, 1050 Brussels, Belgium.

[6] EFSA Dossier reference: FAD-2010-0142.

[7] 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.

[8] Commission Regulation (EC) No 479/2006 of 23 March 2006 as regards the authorisation of certain additives belonging to the group compounds of trace elements. OJ L 86, 24.3.2006, p. 4.

[9] European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003. Available online http://ec.europa.eu/food/food/animalnutrition/feedadditives/comm_register_feed_additives_1831-03.pdf

three zinc-based additives: zinc chelate of amino acids hydrate (EFSA, 2012b; EFSA FEEDAP Panel, 2013b), zinc sulphate monohydrate (EFSA 2012c, 2012d), and zinc oxide (EFSA, 2012e).

Recently, EFSA has issued an opinion on the potential reduction of the currently authorised maximum zinc content in complete feed. The newly proposed maximum contents are: 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).

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall 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 the efficacy of the 'Zinc acetate, dihydrate', 'Zinc chloride, anhydrous', 'Zinc oxide', 'Zinc sulphate, heptahydrate', 'Zinc sulphate, monohydrate', 'Zinc chelate of amino acids, hydrate' and 'Zinc chelate of glycine, hydrate', when used under the conditions described in Table 1.

Table 1: Description and conditions of use of the additive as proposed by the applicant[10]

Additive	Zinc
Registration number/EC No/No (if appropriate)	3b6.xx
Category(-ies) of additive	3. Nutritional additives
Functional group(s) of additive	b. Compounds of trace elements

Description			
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)
Zinc acetate dihydrate ≥ 29.6% Zn	**$Zn(CH_3COO)_2•2H_2O$**	**Complies with EU law on undesirable substances**	**EN 15510:2007 EN ISO 6869:2000**
Zinc chloride anhydrous ≥ 96% Zn	**$ZnCl_2$**		
Zinc oxide. ≥ 72% Zn	**ZnO**		
Zinc sulphate, heptahydrate. ≥ 22% Zn	**$ZnSO_4•7H_2O$**		
Zinc sulphate, monohydrate. ≥ 34 Zinc chelate of amino acids, hydrate ≥ 10% Zn	**$ZnSO_4•H_2O$** **Zn $(x)_{1–3}•nH_2O$ (x=anion of any amino acid derived from hydrolysed soya protein) Molecular weight not exceeding 1500 dalton**		
Zinc chelate of glycine, hydrate ≥ 15% Zn (solid), ≥ 7% Zn (liquid)	**Zn $(x)_{1–3}•nH_2O$ (x= anion of synthetic glycine)**		

[10] Table updated as indicated in the Technical Dossier/Supplementary Information (October 2012).

Trade name (if appropriate)	Not applicable
Name of the holder of authorisation (if appropriate)	TREAC EEIG[11]

Conditions of use for the additives in complete feed (Zinc acetate, dihydrate; Zinc chloride, anhydrous; Zinc oxide; Zinc sulphate, heptahydrate; Zinc sulphate, monohydrate; Zinc chelate of amino acids, hydrate; Zinc chelate of glycine, hydrate (solid) and Zinc chelate of glycine, hydrate (liquid))				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period (if appropriate)
		mg/kg of complete feedingstuffs		
All animal species and categories	-	-	Pet animals: 250 (total) Fish: 200 (total) Milk replacers: 200 (total) Other species: 150 (total)	Not appropriate

Conditions of use for the additives in water for drinking (Zinc acetate, dihydrate; Zinc chloride, anhydrous; Zinc sulphate, heptahydrate; Zinc sulphate, monohydrate; Zinc chelate of glycine, hydrate (solid), Zinc chelate of glycine, hydrate (liquid))				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period (if appropriate)
		mg/litre water for drinking		
All animal species and categories	-	-	Pet animals: 125 (total) Milk replacers: 28.6 (total) Other species: 37.5 - 75 (total)	Not appropriate

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use (if appropriate)	*For the additives to use in feedingstuffs:* The additive shall be incorporated in feeds in form of a premixture. *For the additives to use in water for drinking:* Not appropriate
Specific conditions or restrictions for handling (if appropriate)	For user safety: breathing protection, safety glasses and gloves should be worn during handling.
Post-market monitoring (if appropriate)	Not appropriate (existing feed additive)
Specific conditions for use in complementary feedingstuffs (if appropriate)	- The zinc feed additive is given continuously during animal rearing. - To supply Zn in final feeds with EU legal limits for each species.

Maximum Residue Limit (MRL) (if appropriate)			
Marker residue	Species or category of animal	Target tissue(s) or food products	Maximum content in tissues
Not appropriate	Not appropriate	Not appropriate	Not appropriate

[11] EFSA notes that TREAC EEIG was liquidated on 19/12/2012 and their rights as applicant were transferred to FEFANA asbl

ASSESSMENT

At the time of submission, the application represented 14 companies joint as a Consortium. During the assessment, one company withdrew from the Consortium and therefore its data have not been considered in the current opinion. The opinion is based on data provided by these companies involved in the production/distribution of zinc-containing compounds, and on publicly available literature. It should be recognised that these data cover only a fraction of the existing zinc-based additives available on the market.

1. Introduction

The transition metal zinc is essential to all living organisms. It is an integral component of an estimated 10 % of all proteins, in which it contributes to tertiary structure or catalytic activity covering all enzyme classes. It is also a signalling substance in its functions as second messenger and synaptic neuromodulator. The biological functions of zinc are numerous and diverse and include glucose and lipid metabolism, cell proliferation and embryogenesis, and those related with the nervous and immune systems. The roles of zinc, its deficiency and toxicity symptoms in farm animals have been described in a previous opinion of the Scientific Committee on Animal Nutrition (EC, 2003a), and a brief update on the normal functions and toxicity of zinc is given in Appendix A.

The additives under assessment are: zinc acetate, dihydrate; zinc chloride, anhydrous; zinc oxide; zinc sulphate, heptahydrate; zinc sulphate, monohydrate; zinc chelate of amino acids, hydrate; and zinc chelate of glycine, hydrate (powder and liquid form). These compounds, except the zinc chloride, anhydrous, are already authorised in the European Union (EU) for use in feed. Additionally, the application requests the use of some of the compounds in water for drinking. Therefore, a re-evaluation of authorisation or an authorisation of a new additive, or a new use of an additive, is sought, depending on the compound (see Table 2).

Table 2: Details of the application submitted by the European Commission

Compound	Request	Matrix	
		Feed	Water for drinking
1. Zinc acetate, dihydrate	Re-evaluation	X	
	New use of the additive		X
2. Zinc chloride, anhydrous	New additive		X[12]
3. Zinc oxide	Re-evaluation	X	
4. Zinc sulphate, heptahydrate	Re-evaluation	X	
	New use of the additive		X
5. Zinc sulphate, monohydrate	Re-evaluation	X	
	New use of the additive		X
6. Zinc chelate of amino acids, hydrate	Re-evaluation	X	
7. Zinc chelate of glycine, hydrate: (a) powder or solid or (b) liquid	Re-evaluation	X	
	New use of the additive		X

[12] The applicant stated that this compound would be used only in water for drinking. Technical Dossier/Supplementary Information (October 2012).

A compilation of risk assessments carried out on zinc and its compounds, including opinions from EFSA panels other than the FEEDAP Panel, is given in Appendix B. A list of authorisations of zinc compounds in the EU, other than as feed additives, is reported in Appendix C.

EFSA commissioned the University of Gent (Belgium) to carry out a study of selected trace and ultratrace elements, including zinc. The findings were submitted to the EFSA in the form of a technical report (Van Paemel et al., 2010). Another report on the environmental impact of zinc and copper used in animal nutrition has been provided to EFSA following a call for tender (Monteiro et al., 2010).[13] Information from these reports has been used in this opinion.

An opinion on the revision of the maximum zinc levels authorised in feed was delivered by the FEEDAP Panel (EFSA FEEDAP Panel, 2014). This opinion provided a critical review of the zinc requirements of food-producing and pet animals, the zinc concentration in feed materials and the calculated background zinc concentration of complete feed; it supported the possibility of a considerable reduction in the currently authorised maximum concentrations of total zinc in feed. The newly proposed maximum zinc contents (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) would ensure health, welfare and productivity of the target species, and would result in an overall reduction of zinc emissions from animal production of about 20 %. This opinion will be referred to within the context of the current assessment.

2. Characterisation[14]

For compounds of trace elements, the element itself is considered the active substance.

2.1. Zinc acetate, dihydrate[15]

Characterisation and identity

'Zinc acetate, dihydrate' (Chemical Abstracts Service (CAS) No 5970-45-6) has the chemical formula $Zn(CH_3COO)_2 \bullet 2H_2O$ (molecular weight 219.5 Da, theoretical zinc content 29.8 %).

The product is a solid, white powder with a mild smell of acetic acid. Its solubility in water is 311 g/L at 20 °C and 666 g/L at 100 °C. The pH of a 5 % aqueous solution varies between 6 and 6.6. It has a density of approximately 1.74 g/cm^3 and a bulk density of 0.81 g/cm^3.[16]

The company provided analytical data of five batches showing a zinc content of 29.7–29.9 % (specification ≥ 29.6 %).[17]

Levels of heavy metals (lead (Pb) < 20 mg/kg product, six batches; cadmium (Cd) 0.56–2.3 mg/kg product, four batches; mercury (Hg) < 0.02 mg/kg, one batch), fluorine (F) (< 100 mg/kg, one batch) and arsenic (As) (< 3 mg/kg product, six batches)[18] comply with the thresholds set in Directive 2002/32/EC for compounds of trace elements[19] or, if not mentioned in the Directive, do not represent a concern. Dioxins were analysed in three batches and the sum of dioxins and dioxin-like polychlorinated biphenyls (PCBs) in four batches, showing 0.09 ng WHO-PCDD/F-TEQ/kg and

[13] The FEEDAP Panel notes that this report contains a typographical error when referring to the units of the maximum total copper and zinc authorised in feed (Background and Introduction to the document: it is written mg/*day* and should be mg/*complete feedingstuffs*).
[14] This section has been amended following the confidentiality claims made by the applicant.
[15] One company involved in the application: (d6).
[16] Technical Dossier/Section II/Annex 2.2.12.
[17] Technical Dossier/Section II/Annex 2.1.24.
[18] Technical Dossier/Section II/Annex 2.1.45. Technical Dossier/Supplementary Information (October 2012)/ Annex_Qii_Zn acetate_Cd-dioxins and PCBs.pdf.
[19] 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.

< 0.11–0.124 ng WHO-PCDD/F-PCB-TEQ/kg, respectively;[20] these concentrations comply with those set in Directive 2002/32/EC.

Particle size distribution was characterised in two batches of the product by laser diffraction; the mean particle diameter was determined to be 105 µm and the thoracic fraction (< 50 µm) between 2.2 and 8.7 % (v/v).[21] Data on dusting potential were not provided.

Stability and homogeneity

Three batches were stored in closed polyethylene cans at ambient conditions.[22] The zinc content of the product, analysed by inductively coupled plasma atomic emission spectrometry (ICP–AES), did not change over a period of at least three years, indicating that zinc acetate, dihydrate, is stable. No data on stability of the compound in premixtures and compound feed were provided.

To examine the capacity of zinc acetate, dihydrate, to homogeneously distribute in premixtures, 1488 mg of zinc, from zinc acetate, dihydrate, were added per kilogram of premixture for piglets.[23] The analysis of the total zinc content of ten samples provided a coefficient of variation (CV) of 7.5 %.

Homogeneity according to theoretical calculations (Jansen, 1992) was also provided for piglet compound feed at inclusion rate of 500 mg Zn/kg, resulting in a CV of 2.44 %.[24] However, this method has been developed to test the working accuracy of mixing equipment and it is not accepted by the FEEDAP Panel as a valid method for assessing the homogeneity of distribution of additives in feeds.

Manufacturing process

Zinc acetate is formed by reacting zinc oxide with acetic acid. The zinc acetate, dihydrate, is crystallised, sifted and dried. The final product is monitored by routine quality control measures.

2.2. Zinc chloride, anhydrous[25]

Characterisation and identity

'Zinc chloride, anhydrous' (CAS No 7646-85-7) has the chemical formula $ZnCl_2$ (molecular weight 136.27 Da, theoretical zinc content 48.0 %).

The product is a solid, white to slightly coloured powder, odourless. Its solubility in water is of 432 g/L at 25 °C or 614 g/L at 100 °C. The pH is 3.5 in a 50 % aqueous solution. It has a density of approximately 1.4 g/cm^3 and a bulk density of 0.149 g/cm^3.[26]

The company provided analytical data of five batches showing a zinc content of 46.1–47.0 % (specification ≥ 46.1 %).[27]

The product is highly hygroscopic and therefore is intended for use in water for drinking only.

Levels of heavy metals (Pb < 0.05–2.9 mg/kg product, three batches; Cd 0.4–1.38 mg/kg product, three batches; Hg < 0.05 mg/kg product, two batches), F (14.5–29 mg/kg product, three batches) and

[20] Technical Dossier/Section II/Annex 2.1.67. Technical Dossier/Supplementary Information (October 2012)/Annex_Qii_Zn acetate_Cd-dioxins and PCBs.pdf.
[21] Technical Dossier/Section II/Annex 2.2.32.
[22] Technical Dossier/Section II/Annex 2.4.2.
[23] Technical Dossier/Section II/Annex 2.4.7.
[24] Technical Dossier/Section II.
[25] One company involved in the application: (d10).
[26] Technical Dossier/Section II/Annex 2.2.13.
[27] Technical Dossier/Section II/Annex 2.4.2.

As (< 0.05 mg/kg product, three batches)[28] comply with the thresholds set in Directive 2002/32/EC for compounds of trace elements or, if not mentioned in the Directive, do not represent a concern. Dioxins and the sum of dioxins and dioxin-like PCBs were analysed in three batches showing 0.09–0.64 ng WHO-PCDD/F-TEQ/kg and 0.125–0.71 ng WHO-PCDD/F-PCB-TEQ/kg, respectively;[29] these concentrations comply with those set in Directive 2002/32/EC.

Particle size distribution was characterised in three batches of the product; all particles had a diameter of > 100 µm (technique used not indicated).[30] Data on dusting potential were not provided.

Stability and homogeneity

Stability data are not required for inorganic compounds of trace elements.

Because of the high solubility in water of the compound, no further demonstration of homogeneity is deemed necessary.

Manufacturing process

Zinc chloride is formed by reacting zinc oxide with hydrochloric acid. The zinc chloride solution is concentrated, filtered and dried. The final product is monitored by routine quality control measures.

2.3. Zinc oxide[31]

Characterisation and identity

'Zinc oxide' (CAS No 1314-13-2) has the chemical formula ZnO (molecular weight 81.34 Da, theoretical zinc content 80.4 %).

The product is a solid, white to dark green or beige/brownish powder, odourless. It has a very low solubility in water (1.6 mg/L at 20 °C). The pH is reported to be between 7 and 9.5.[32] It has a density of 5.6 g/cm^3 and a bulk density ranging from 0.8 to 2.5 g/cm^3.[33,34]

The measured zinc content of the product, in a total of 19 batches (4 or 5 batches/company), was 72–80 % (specification ≥ 72 %). For further details regarding characterisation and identity of zinc oxide, see Appendix D, Table 4.

Levels of heavy metals (Pb 7−343 mg/kg product; Cd < 0.1–12 mg/kg product) and As (0.6–61 mg/kg product), analysed in three to eight batches of the product from each company, comply with the thresholds set in Directive 2002/32/EC for compounds of trace elements. Levels for Hg (< 10 mg/kg, three to five batches from two companies) do not represent a concern. Analyses of dioxins (one to six batches/company) and the sum of dioxins and dioxin-like PCBs (one to six batches from three companies) were carried out; concentrations (0.044–0.20 ng WHO-PCDD/F-TEQ/kg and 0.086–0.28 ng WHO-PCDD/F-PCB-TEQ/kg, respectively) comply with those set in Directive 2002/32/EC.

[28] Technical Dossier/Section II/Annex 2.1.46.
[29] Technical Dossier/Section II/Annex 2.1.68 and Technical Dossier/Supplementary Information (October 2012)/Annex_Qiii_Zn chloride_dioxins and PCBs.pdf.
[30] Technical Dossier/Section II/Annex 2.2.33.
[31] Four companies involved in the application: (d2), (d5), (d7) and (d14)..
[32] The FEEDAP Panel notes the high pH indicated for the product of some companies and the overall variability of the pH reported across the companies. This might be as a result of the source of the zinc oxide: the crushed zinc dolomite; this source contains magnesium hydroxide/carbonate.
[33] Technical Dossier/Section II/Annex 2.2.14 (d2), Annex 2.2.15 (d5), Annex 2.2.16 (d7), Annex 2.2.17 (d11), Annex 2.2.18 (d14).
[34] The high variability of the bulk density might be caused by the variability in particle size and different flowability of the products of the various companies.

Particle size distribution was characterised in one to five batches from each company (by laser diffraction or sieving); 17–95 % (v/v) of the particles had a diameter of < 52.2 µm, and up to 36 % of the particles had a diameter of < 10.5 µm. Stauber–Heubach analysis of a single batch of the product with the highest fraction of particles with diameter < 51 µm, indicated a dusting potential of 0.19 g/m^3.

Stability and homogeneity

Stability data are not required for inorganic compounds of trace elements.

No experimental data on the homogeneous distribution of the additive were provided. Instead, the theoretical Jansen method (Jansen, 1992) was applied to estimate the homogeneity of the product from company d14 in premixes[35] and from company d7 in broiler feed;[36] the calculated CV (with a 68 % probability) for ZnO ranged from 0.002 to 0.004 % in different premixes and was 2.1 % in broiler feed. However, this method has been developed to test the working accuracy of mixing equipment and it is not accepted by the FEEDAP Panel as a valid method for assessing the homogeneity of distribution of additives in feeds.

Manufacturing process

Zinc oxide is sourced from crushed zinc dolomite. The crushed ore is calcinated and zinc is lixiviated using ammonium bicarbonate. Various purification steps are deployed to remove metal impurities. A steam precipitation of zinc carbonate is performed, and the zinc carbonate is warmed to remove CO_2 and turn it into zinc oxide. The final product is analysed by routine quality control measures, in accordance with methods of specification based on feed hygiene regulations. If all tests comply with the defined limits, the material is released for use after packaging.

2.4. Zinc sulphate, heptahydrate[37]

Characterisation and identity

'Zinc sulphate, heptahydrate' (CAS No 7446-20-0) has the chemical formula $ZnSO_4 \cdot 7H_2O$ (molecular weight 287.55 Da; theoretical zinc content 22.7 %).

The product is an odourless white powder with solubility in water of 960 g/L at 20 °C. The pH is of 4–6. It has a density of 1.97 g/cm^3 and a bulk density of 1.12 g/cm^3 (average of five samples).[38]

The zinc content of the product, measured in two batches, was reported to be between 23.9 %[39] and 24.2 %[40] (specification ≥ 22 %). It is noted that the measured content slightly exceeds the theoretical maximum zinc content and the FEEDAP Panel assumes that this discrepancy is caused by imprecision in the analytical methods used.

Levels of heavy metals (Pb 5.8–6.1 mg/kg product; Cd < 0.1 mg/kg product; Hg < 0.3 mg/kg) and As (< 0.1 mg/kg product), each analysed in three batches of the product,[41] comply with the thresholds set in Directive 2002/32/EC for compounds of trace elements or, if not mentioned in the Directive, do not represent a concern. Dioxins and dioxin-like PCBs were analysed in two batches showing 0.074 ng WHO-PCDD/F-TEQ/kg and 0.062 ng WHO-PCBs-TEQ/kg, respectively;[42] the concentrations of dioxins comply with those set in Directive 2002/32/EC. Data on the sum of dioxins plus dioxin-like PCBs were not provided.

[35] Technical Dossier/Section II/Annex 2.4.12.
[36] Technical Dossier/Section II/Annex 2.4.13.
[37] One company involved in the application: (d9).
[38] Technical Dossier/Section II/Annex 2.2.19.
[39] Technical Dossier/Supplementary Information (October 2012)/Annex_Qviii_Zn sulphate_7H2O_batch to batch.pdf.
[40] Technical Dossier/Section II/Annex 2.1.31.
[41] Technical Dossier/Section II/Annex 2.1.52.
[42] Technical Dossier/Section II/Annex 2.1.74.

Particle size distribution was characterised for two batches of the product by laser diffraction;[43] 0.8–2.1 % (v/v) of the particles had a diameter of < 50 µm, whilst 0–0.1 % (v/v) had a diameter of < 10 µm. Dusting potential in the product (data from one batch, Stauber-Heubach method) could not be detected.[44]

Stability and homogeneity

Stability data are not required for inorganic compounds of trace elements.

No experimental data on the homogenous distribution of the additive were provided. Instead, the theoretical Jansen method (Jansen, 1992) was applied to estimate the homogeneity of the product in four animal feeds;[45] the calculated CV for $ZnSO_4 \bullet 7H_2O$ ranged from 2.3 %, in a milk replacer (calculated from an inclusion level of 700 mg Zn/kg[46]), to 8.4 %, in a broiler feed. The Panel notes that the inclusion level is 3.5-fold higher than that allowed by legislation. However, this method has been developed to test the working accuracy of mixing equipment and it is not accepted by the FEEDAP Panel as a valid method for assessing the homogeneity of distribution of additives in feeds.

Manufacturing process

To produce zinc sulphate, heptahydrate, zinc oxide is first mixed with sulphuric acid. Various purification steps are deployed to remove metal impurities. Clean zinc sulphate solution is concentrated in evaporators and then crystallised.

2.5. Zinc sulphate, monohydrate[47]

Characterisation and identity

'Zinc sulphate, monohydrate' (CAS No 7446-19-7) has the chemical formula $ZnSO_4 \cdot H_2O$ (molecular weight 179.46 Da, theoretical zinc content 36.4 %).

The product is a solid, white to cream coloured odourless powder. Its solubility in water is of 400 g/L at 20 °C. The pH is of 5–6 (50 g/L water as suspension, 20 °C). The product has a density of 3.2 g/cm^3,[48] and a bulk density of 0.8–1.25 g/cm^3.[49]

The zinc content of the product, measured in five batches, ranged from 34 % to 36 % (specification ≥ 34 %).[50]

Levels of heavy metals (Pb 7–29 mg/kg product; Cd 3–9 mg/kg product; eight batches each) and As (< 10 mg/kg product, four batches)[51] comply with the thresholds set in Directive 2002/32/EC for compounds of trace elements. Dioxins and the sum of dioxins plus dioxin-like PCBs were analysed in four and one batches, respectively, showing 0.017–0.065 ng WHO-PCDD/F-TEQ/kg and 0.016 ng WHO-PCDD/F-PCB-TEQ/kg, respectively;[52] these concentrations comply with those set in Directive 2002/32/EC.

[43] Technical Dossier/Section II/Annex 2.2.39.
[44] Technical Dossier/Supplementary Information (October 2012)/Annex_Qviii_Zn sulphate_7H_2O_Dusting potential.pdf
[45] Technical Dossier/Section II.
[46] The FEEDAP Panel notes that the supplementation level tested exceeds the maximum authorised zinc content for milk replacer.
[47] One company involved in the application: (d2) Arkop.
[48] Technical Dossier/Section II/Annex 2.5.21.
[49] Technical Dossier/Section II/Annex 2.2.20.
[50] Technical Dossier/Section II/Annex 2.1.32.
[51] Technical Dossier/Section II/Annex 2.1.32 (for Pb, Cd and As) and Annex 2.1.53 (for Pb and Cd).
[52] Technical Dossier/Section II/Annex 2.1.75 and Technical Dossier/Supplementary Information (October 2012)/Annex_Qxi_Zn sulphate_1H2O_ Dioxins and PCBS.pdf.

Particle size distribution was characterised for one batch of the product by laser diffraction;[53] 64.5 % (v/v) of the particles had a diameter of < 50 µm and 20 % (v/v) had a diameter of < 10 µm. Dusting potential (Stauber-Heubach method) of one batch was 8.2 g/m^3.[54]

Stability and homogeneity

Stability data are not required for inorganic compounds of trace elements.

No experimental data on the homogenous distribution of the additive were provided. Instead, the theoretical Jansen method was applied to estimate homogeneity of the product in a 'lactating cow feed';[55] the calculated CV for $ZnSO_4 \bullet H_2O$ in this feed was 0.01 % (calculated from an inclusion level of 419 mg Zn/kg). However, this method has been developed to test the working accuracy of mixing equipment and it is not accepted by the FEEDAP Panel as a valid method for assessing the homogeneity of distribution of additives in feeds.

Manufacturing process

The production of zinc sulphate, monohydrate, starts with an unrefined zinc sulphate solution. The zinc sulphate solution is crystallised, weighted and packaged following a series of additional quality control points.

2.6. Zinc chelate of amino acids, hydrate[56]

Characterisation and identity

'Zinc chelate of amino acids, hydrate' is described by the applicant with the generic chemical formula $Zn(x)_{1–3} \bullet nH_2O$, where x= anion of any amino acid derived from hydrolysed soybean protein, molecular weight not exceeding 1500 Da.[57]

The product is a solid, beige to dark tan-coloured powder with a slight characteristic odour. It is reported to be insoluble in water. The product has a density of 1.3 g/cm^3 and the bulk density ranges from 0.4 to 0.75 g/cm^3.[58]

The zinc content of 21 batches of the additive (three to five batches from each company) ranged from 12.3 to 16.6 % (specification ≥ 10 %). The applicant was requested to provide data on the proportions of zinc chelate and any inorganic zinc. The applicant developed an indirect method (based on Fourier Transform Infrared (FTIR) spectroscopy) to estimate the binding of zinc to amino acids. The values were quantified by comparison with a calibration curve. The predicted degree of chelation for the products (average of three to five batches of the product from five companies) ranged from 88 to 114 %. The results of this *in-house* method, although validated, are not considered fully reliable but allow the conclusion that at least 85 % of total zinc occurs as chelates.

Analytical data was provided for the products of four companies showing the percentage of the molecules with a molecular weight < 1500 Da. Considering all values, up to 43 % of the molecules exceeded 1500 Da. The FEEDAP Panel notes that the specification of the applicant on the molecular

[53] Technical Dossier/Section II/Annex 2.2.40.
[54] Technical Dossier/Supplementary Information (October 2012)/Annex_Qxii_Zn sulphate_1H2O_Dusting potential.pdf.
[55] Technical Dossier/Section II/Annex 2.4.14.
[56] Seven companies involved in the application: (d1) Alltech, (d2) Arkop, (d3) Balchem (d4) Biochem, (d7) Orffa, (d8) Pancosma and (d13) Trouw Nutrition International. During the course of the evaluation, the applicant notified that companies d3. Balchem and d7. Orffa are no longer defending this additive within the current application; no Supplementary Information was provided by those companies and therefore they were disregarded from the relevant assessment.
[57] 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.
[58] Technical Dossier/Section II/Annex 2.2.1 (d1), Annex 2.2.2 (d2), Annex 2.2.4 (d4), Annex 2.2.6 (d8), Annex 2.2.7 (d13).

weight of the compound 'not exceeding 1500 Da' is not met by analytical data, and does not comply with current legislation (Commission Regulation (EC) No 1334/2003).

The applicant provided the analysis of five batches of the hydrolysate material before the addition of the zinc source for free and total amino acids; the lysinoalanine content was also analysed showing to be below 50 mg/100 g.

Proximate analysis of a total of seven batches (one, two or three/company) of the additive revealed a content of 4.7–9.1 % moisture, and on an *as is* basis, 26.5–33.6 % crude protein (nitrogen × 6.25), 0.5–1.5 % lipids, 0.4–2.7 % crude fibre and 21.1–32.5 % ash; the mineral fraction consists of 0.2–0.7 % calcium, 2.0–8.3 % sulphur, 0.2–0.8 % sodium, 1.1–1.5 % potassium and 0.2–0.5 % phosphorus.[59,60,61,62,63,64] For further details regarding characterisation and identity of zinc chelate of amino acids, hydrate, see Appendix D, Tables 5 and 6.

Levels of heavy metals (Pb < 0.1–24 mg/kg and Cd 0.42–5 mg/kg, analysed in at least three batches from each company; Hg < 0.005–0.5 mg/kg, three batches of the product from four companies), F (48–60 mg/kg, three batches of the product from one company) and As (< 0.10–2.3 mg/kg, at least one batch/company) comply with the thresholds set in Directive 2002/32/EC for compounds of trace elements, or if not mentioned in the Directive, do not represent a concern. Dioxins (analysed in a total of 18 batches from five companies) and the sum of dioxins and dioxin-like PCBs (analysed in a total of 14 batches from the five companies) showed to be 0.004–0.19 ng WHO-PCDD/F-TEQ/kg and 0.004–0.150 ng WHO-PCDD/F-PCB-TEQ/kg, respectively; these concentrations comply with those set in Directive 2002/32/EC.

The analysis of mycotoxins (aflatoxin B1 and ochratoxin A) in nine batches of the product from five companies did not raise concern (aflatoxin B1 was below 5 µg/kg; ochratoxin A was found at maximum 1 µg/kg additive). In the batches stored for 3 to 38 months, the content of moulds, yeasts and total coliforms was below 100 CFU/g; *Salmonella* was absent in 25 g.

Particle size distribution was characterised in one to four batches per company (by laser diffraction or sieving). The fraction of particles with diameter below 63 µm ranged from 3.1 % to 70.1 % (data from five companies), and that below 11 µm ranged from 0 % to 4.5 % (data from four companies). Dusting potential (Stauber-Heubach method) was determined only in the product identified as having the highest percentage of particles < 45.4 µm and was 0.97 g/m³ (average of four batches).

Stability and homogeneity

No stability (including shelf-life) data were provided for the zinc chelate of amino acids, hydrate, in particular concerning the maintenance of the specific bonds of zinc in the chelates. The FEEDAP Panel recognises the analytical difficulties to demonstrate stability of these specific bonds and notes that the active substance is also unlikely to disappear in these products.

The product showed to be stable to microbial contamination for 3 to 38 months (see Characterisation and identity).

[59] Technical Dossier/Supplementary Information (October 2012)/COMPANY (D1). Annex_Qii_TREAC_ COMPANY (D1)_ZINC_composition1.pdf.
[60] Technical Dossier/Supplementary Information (October 2012)/COMPANY (D2). COMPANY (D2) Zn AA chelate data Q(ii) .pdf.
[61] Technical Dossier/Supplementary Information (October 2012)/COMPANY (D4). Annexes. Annex_Qiii_TREAC_ COMPANY (D4)__ZINC_composition1.pdf.
[62] Technical Dossier/Supplementary Information (October 2012)/COMPANY (D4). Annexes. Annex_Qiii_TREAC_ COMPANY (D4)__ZINC_composition2.pdf.
[63] Technical Dossier/Supplementary Information (October 2012)/COMPANY (D8). Annex_Qiii_TREAC_COMPANY (D8) _ZINC_composition1.pdf.
[64] Technical Dossier/Supplementary Information (October 2012)/Company (d13).Annex_Qii_TREAC_Company (d13) _ZINC_Composition.pdf.

Experimental data on the capacity for homogeneous distribution of the additive were provided by company d1. Zinc content was measured in 15 samples of a premixture for a sow feed (average 23.5 g Zn/kg premixture)[65] and in 14 samples of a complementary feed for horses (average 685 mg Zn/kg feed).[66] The CVs for the premixture and the complementary feed were 0.4 % and 1.6%, respectively. Additionally, company d8 provided a study to demonstrate the homogeneity of several compounds of zinc, including zinc chelate of amino acids, hydrate, in a mineral premixture; the CV was about 1.5 %.[67]

In addition, the theoretical Jansen method (Jansen, 1992) was applied to estimate the homogeneity of the additive in a 'starter feed for broilers'; the calculated CV for the zinc in the tested feed was 0.11 % (calculated from an inclusion level of 150 mg Zn/kg). However, this method has been developed to test the working accuracy of mixing equipment and it is not accepted by the FEEDAP Panel as a valid method for assessing the homogeneity of distribution of additives in feeds.

Manufacturing process

The manufacturing process starts with enzymatic hydrolysis of soybean protein (under specific pH conditions). The hydrolysation process is followed by chelation with a source of zinc. The slurry is dried. The applicant confirmed that only unblended products are placed on the EU market by the companies participating in this application since no anticaking/carrier or other diluents are added.[68] The applicant provided a generic process flow chart.

The applicant stated that all the ingredients—including enzymes—used to manufacture the additive are derived from genetically modified organisms (GMOs).[69]

2.7. Zinc chelate of glycine, hydrate

'Zinc chelate of glycine, hydrate' is derived from synthetic glycine mixed with a zinc salt. It has the generic formula Zn $(x)_{1–3}$•nH_2O, where x= anion of glycine.

The application is for the additive in two forms, solid and liquid.

2.7.1. Zinc chelate of glycine, hydrate (solid)[70]

Characterisation and identity

The product is a white or cream-coloured odourless powder. Its solubility in water is in the range 10 to 250 g/L. The product has a bulk density ranging from 0.8 to 1.03 g/cm^3.[71]

The zinc content of 21 batches of the additive, at least one batch from each company, ranged from 15.6 to 30.9 %—the large range results primarily from the different ratios of zinc to glycine (specification ≥ 15 %). For further details regarding characterisation and identity of zinc chelate of amino acids, hydrate, see Appendix D, Table 7.

Upon EFSA's request, the applicant provided data on the composition of a total of ten batches of the zinc compound.[72] The zinc content ranged from 15.8 to 27.3 %; extractable glycine, from 29.9 to 64.3 %; sulphur, from 0.1 to 13.8 % and moisture from 1.1 to 9.6 %. The molar ratio of glycine to zinc in the different products ranged from 3.2 to 1. (See also Appendix D, Table 8).

[65] Technical Dossier/Section II/Annex 2.4.8.
[66] Technical Dossier/Section II/Annex 2.4.10.
[67] Technical Dossier/Section II/Annex 2.4.6a.
[68] Technical Dossier/Supplementary Information (October 2012).
[69] Technical Dossier/Supplementary Information (October 2012).
[70] Four companies involved in this application: (d2), (d4), (d8) and (d12).
[71] Technical Dossier/Section II/Annex 2.2.8 (d2), Annex 2.2.9 (d4), Annex 2.2.10 (d8), Annex 2.2.11 (d12).
[72] Technical Dossier/Supplementary Information (January 2015).

Levels of heavy metals (Pb < 0.5–28 mg/kg, Cd < 1–4 mg/kg, at least three batches/company; Hg < 0.0007–< 1mg/kg, at least three batches from three companies) and arsenic (< 0.01–8 mg/kg, at least three batches/company) comply with the thresholds set in Directive 2002/32/EC for compounds of trace elements or, if not mentioned in the Directive, do not represent a concern. Dioxins (analysed in a total of ten batches from the five companies) and the sum of dioxins and dioxin-like PCBs (analysed in a total of three batches from two companies) showed 0.009–0.17 ng WHO-PCDD/F-TEQ/kg and < 0.01–0.071 WHO-PCDD/F-PCB-TEQ/kg, respectively; these concentrations comply with those set in Directive 2002/32/EC.

Particle size distribution was characterised in one to three batches per company by laser diffraction. The products differed markedly in their particle size distribution. The fraction of particles with diameter < 56 µm ranged from 0.33 % to 44.6 %, and with diameters of < 10 µm ranged from 0 % to 6.6 %. Dusting potential (Stauber-Heubach method) was determined only in one batch of the product identified as having the highest percentage of particles < 50 µm and was 1.7 g/m³.

Stability and homogeneity

No stability (including shelf-life) data were provided for the zinc chelate of glycine, hydrate (solid), in particular concerning the maintenance of the specific bonds of zinc in the chelate. The FEEDAP Panel recognises the analytical difficulties to demonstrate stability of this specific bound and notes that the active substance is also unlikely to disappear in these products.

The homogeneous distribution of zinc chelate of glycine, hydrate (solid), from one company (out of four) was examined experimentally in 15 samples of a mineral premixture (3.2 g Zn/kg premixture)[73] and in nine samples of a non-specified target species complete feed (44.3 mg Zn/kg feed).[74] The CVs for the premixture and the complete feed were 3.6 % and 5.6%, respectively.

Manufacturing process

To produce zinc chelate of glycine, hydrate (solid), a zinc salt is mixed with synthetic glycine. The raw materials could be of any source that complies with EU Regulations; the FEEDAP Panel gives consideration to the fact that the source of the zinc salt will influence the composition of the product (e.g. sulphate will be retained if zinc sulphate is used for the starting reaction). The chelation reaction conditions are controlled and monitored. Then the product is dried. The applicant confirmed that only unblended solid products are placed on the EU market by the companies participating in this application.

2.7.2. Zinc chelate of glycine, hydrate (liquid)[75]

To produce the additive, zinc sulphate monohydrate is mixed in water with an equimolar concentration of synthetic glycine (complying with EU Regulations). Sodium benzoate and potassium sorbate are added to the solution, which is then filtered and treated with ultraviolet (UV) light.

Characterisation and identity

The product is an odourless, transparent and colourless liquid. The zinc content of three batches of the additive was 7.2–7.8% (≥ 7 % specification).[76]

Levels of heavy metals (Pb 0.033–0.055 mg/kg product, Cd: < 0.025 mg/kg product, two batches) and As (< 0.025 mg/kg product, two batches)[77] comply with the thresholds set in Directive 2002/32/EC for

[73] Technical Dossier/Section II/Annex 2.4.9.
[74] Technical Dossier/Section II/Annex 2.4.11.
[75] One company involved in the application: (d8).
[76] Technical Dossier/Section II/Annex 2.1.22b.
[77] Technical Dossier/Section II/Annex 2.1.54.

compounds of trace elements. Dioxins and dioxin-like PCBs were analysed in two batches each, showing 0.34–0.69 ng WHO-PCDD/F-TEQ/kg and 0.23 ng WHO-PCBs-TEQ/kg product, respectively;[78] concentrations for dioxins comply with those set in Directive 2002/32/EC. Data on the sum of dioxins plus dioxin-like PCBs were not provided.

Microbial contamination was analysed in one batch at 0, 24 and 48 hours after dilution in water.[79] At all time points studied, total aerobic count, moulds and yeasts were below 1 CFU/g, and *Salmonella* was not detected in 25g sample of the product. However, it appears that this study may have not been performed under practical farm conditions.

Stability and homogeneity

No stability (including shelf-life) data were provided for the zinc chelate of glycine hydrate (liquid), in particular concerning the maintenance of the specific bonds of zinc in the chelates. The FEEDAP Panel recognises the analytical difficulties to demonstrate stability of these specific bonds and notes that the active substance is also unlikely to disappear in these products.

2.8. Physico-chemical incompatibilities in mixtures

Based on current knowledge, no incompatibilities resulting from the use of zinc in compound feed are expected, other than those widely known and considered by feed manufacturers in diet formulation.

The applicant provided data on the stability of vitamins A, D_3 and E in a liquid vitamin–mineral mixture as influenced by zinc chelate of glycine, hydrate, or zinc sulphate monohydrate (10 mg Zn/L) also containing iron, manganese and copper. The vitamin concentration in this mixture was compared to a control mixture (without addition of trace minerals) after two weeks of storage. The three vitamins appeared stable in the presence of the zinc sources tested (and also the other trace elements).[80]

2.9. Conditions of use

Six of the zinc compounds under application (zinc acetate, dihydrate; zinc sulphate, heptahydrate; zinc sulphate, monohydrate; zinc oxide; zinc chelate of amino acids, hydrate; zinc chelate of glycine, hydrate) are intended to be mixed into feedingstuffs via a premixture.

Four compounds (zinc acetate, dihydrate; zinc sulphate, heptahydrate; zinc sulphate, monohydrate; zinc chelate of glycine, hydrate) are also intended for use in water for drinking.

One compound (zinc chloride anhydrous) is intended to be used only in water for drinking. Upon EFSA's request, the applicant clarified that the zinc chloride, anhydrous, is distributed only after dilution in water, as a liquid formula.

For details on the concentrations proposed for use in feed or water, see Table 3.

Table 3: Maximum contents of zinc in complete feed and water for drinking, as proposed by the applicant.

Animal species/category	Maximum total Zinc content	
	Complete feed (mg/kg)	Water for drinking (mg/L)*
Pet animals	250	125
Fish	200	
Other species	150	37.5–75
(Use in milk replacers)	200	28.6

(*) The proposed zinc content in water assumes that no zinc is administered via feed (including feed background).

[78] Technical Dossier/Section II/Annex 2.1.76.
[79] Technical Dossier/Supplementary Information (October 2012)/Annex_Qxxiii and Qxxiv_Zn glycinate_stability water_microbio.pdf.
[80] Technical Dossier/Section II/Annexes 2.4.18a and 2.4.18b.

The use of certain zinc compounds (zinc acetate, dihydrate; zinc chloride, anhydrous; zinc sulphate, heptahydrate; zinc sulphate, monohydrate; zinc chelate of glycine, hydrate (solid and liquid)) in water for drinking is requested for all animal species and categories, except fish. The maximum zinc contents proposed for water for drinking are derived from the maximum contents set for complete feed by dividing by a factor (based on the ratios of the intakes of water to feed) of 2 for pet animals, of 7 for milk replacers and of 2–4 for other species. The resulting maximum concentrations are 125 mg Zn/L for pet animals, 28.6 mg Zn/L for use in milk replacers and 37.5–75 mg Zn/L for other species.[81,82] It is noted that these calculations for the use of zinc in water for drinking do not consider the background zinc content in feed.

In 2010, the FEEDAP Panel recommended that compounds of trace elements should generally not be used via water for drinking (EFSA, 2010).

2.10. Evaluation of the analytical methods by the European Union Reference Laboratory (EURL)

EFSA has verified the EURL report as it relates to the methods used for the control of zinc (seven compounds, including: zinc acetate, dehydrate; zinc sulphate, monohydrate; zinc sulphate, heptahydrate; zinc chloride, anhydrous; zinc oxide; zinc chelate of amino acids, hydrate; zinc chelate of glycine, hydrate) in animal feed. The Executive Summary of the EURL report can be found in the Annex A.

3. Safety

3.1. Safety for the target species

No specific studies on tolerance of target species/categories with the compounds under assessment were submitted.

Zinc is recognised to be of low to moderate oral toxicity in farm animals. The National Research Council (NRC, 2005) evaluated the maximum tolerable zinc dietary concentrations in several animal species and found these to be 250 mg/kg for fish, 300 mg/kg for sheep, 500 mg/kg for cattle and poultry, and 1000 mg/kg for pigs. By interspecies extrapolation, the NRC derived maximum tolerable concentrations of 500 mg/kg for equine species and rodents. The limited data available for pets allow only a conservative approximation of the maximum tolerable dietary level, which may be 500 mg/kg diet. Regarding the tolerance level for fish, the FEEDAP Panel notes that literature reports markedly different values for different species, i.e. < 100 mg/kg for tilapia (*Oreochromis niloticus*) and > 2000 mg/kg for carp (several species) and rainbow trout (*Oncorhynchus mykiss*) (Clearwater et al., 2002).

Given the currently authorised maximum total zinc levels in complete feed, the margin of safety (maximum tolerable concentration/maximum content of zinc authorised in feed) varies between species, as follows: 1.25 for fish, 2 for sheep, 3.3 for cattle and poultry, and 6.7 for pigs. For the species with incomplete datasets, the margin of safety is about 3 for equine species and rodents, and about 2 for pets. In its recently adopted Opinion, the FEEDAP Panel proposed new maximum zinc content in complete feeds which could replace the current ones and thus result in higher margins of safety in all species/categories (up to 30 %) (EFSA, FEEDAP Panel, 2014).

The use of the zinc compounds under application at the maximum authorised levels in feed is considered safe also regarding potential differences in the bioavailability of the different zinc compounds and a higher availability of background zinc because of the widespread use of phytases. Also zinc chloride, anhydrous, which is not currently authorised in the EU and would replace the formerly used zinc chloride, monohydrate, is included in this conclusion.

[81] Technical Dossier/Supplementary Information (October 2012)/Annex_Qii_use in feed and water.pdf.
[82] Technical Dossier/Supplementary Information (October 2012)/Annexes and References to use in feed and water.

No legal maximum concentrations are established for the use of zinc additives in water for drinking. The concentrations of zinc for use in water for drinking must guarantee that the total daily intake of zinc does not exceed those based on zinc in feedstuffs alone when provided at the maximum authorised levels. To derive maximum safe levels in water for drinking, the intake ratio of water to feed, for the different animal species/categories and feedingstuffs, has to be considered, as well as the background zinc content of feedingstuffs. As conservative estimates for the intake water/feed, 2 is taken for pets, 2.5 for poultry, 3 for pigs and 8 for milk replacers. Since the water to feed intake ratio for ruminants cannot be established, the maximum zinc intake for cattle and dairy cows should be expressed as a daily dose. As default value for background zinc content in feedingstuffs, 50 mg/kg is taken, although the range, 20–70 mg Zn/kg, is wide (EFSA FEEDAP Panel, 2014). As a result, the following maximum concentrations of zinc in water for drinking are considered safe, providing that the simultaneous administration of zinc-supplemented via feed and water is avoided: milk replacers, 20 mg/L; pigs, 30 mg/L; poultry, 40 mg/L; pets 100 mg/L. The maximum daily amount of zinc administered via water for drinking to cattle should be 500 mg and to dairy cows 2000 mg. These figures are based on the levels currently authorised in feed and would consequently need a reduction if the newly proposed maximum zinc contents (EFSA FEEDAP Panel, 2014) were adopted by the European Commission. The FEEDAP Panel reiterates that the above-calculated maximum concentrations/doses of zinc supplementation of water for drinking for target species are only safe if the simultaneous use of both feed and water supplemented with zinc is avoided.

3.1.1. Conclusions on the safety for the target species

The FEEDAP Panel concludes that all the zinc compounds under application (zinc acetate, dihydrate; zinc chloride, anhydrous; zinc oxide; zinc sulphate, heptahydrate; zinc sulphate, monohydrate; zinc chelate of amino acids, hydrate; zinc chelate of glycine, hydrate) are safe sources of zinc for all animal species/categories when used up to maximum EU-authorised zinc levels in complete feed. The simultaneous use of both feed and water supplemented with zinc should be avoided.

3.2. Safety for the consumer

The toxicological properties of zinc have been discussed in detail by the Scientific Committee on Food (SCF) (EC, 2003b). It exerts low oral acute toxicity, with high doses (2–8 mg/kg body weight per day in different species) resulting in gastrointestinal distress with clinical signs of nausea, vomiting, abdominal cramps and diarrhoea. Some positive results were observed in genotoxicity tests. In the opinion of the SCF, the weight of evidence from the *in vitro* and *in vivo* genotoxicity tests supports the conclusion that zinc, notwithstanding some positive findings at chromosome levels with elevated doses, has no biologically relevant genotoxic activity (reviewed by the World Health Organization (WHO, 2001)). This conclusion is supported by the US Agency for Toxic Substances and Disease Registry (ATSDR, 2005). The toxicology of zinc has been recently reviewed by Nordberg et al. (2015).

One of the most sensitive and well-described effects of chronic excess zinc intake is a depressed copper uptake with associated copper deficiency effects (reviewed by Maret and Sandstead, 2006).

The SCF derived tolerable upper intake level (UL) of 25 mg/day for adults, and of 13 mg/day and 7 mg/day for 7–10 years children and 1–3 years toddlers, respectively (EC, 2003b). The UL was based on a depressed copper uptake and an altered lipid profile in humans. An uncertainty factor of 2 was applied owing to the small number of subjects included in relatively short-term studies but acknowledging the rigidly controlled metabolic experimental conditions employed.

3.2.1. Absorption and deposition

The metabolic behaviour of zinc was discussed in detail by the SCF (EC, 2003b) and, more recently, by the FEEDAP Panel (EFSA FEEDAP Panel, 2014). Intestinal zinc absorption is saturable, and the rate of zinc uptake is inversely proportional to intake within its large range of homeostatic regulation. Therefore, supplementation of zinc above its requirements and within the authorised levels does not

substantially change zinc deposition in edible tissues, except in liver and kidney, where limited increases in deposition can be observed with increased intake.

Potential differences in deposition from different sources of trace element among tissues and other products used for human consumption play a central role in the assessment of consumer safety. A particular concern is the potential differences in tissue distribution patterns of trace elements from inorganic and organic sources. The applicant provided a report on relevant publications comparing organic and inorganic zinc compounds,[83] covering also copper, iron and manganese, in which a total of 50 experiments performed with zinc (32 with zinc alone and 18 combined with other minerals) were identified; Table 9 in Appendix E summarises the data from these studies on zinc deposition in liver or muscle, resulting from organic and inorganic zinc supplementation to animals. Four experiments compared zinc concentrations in muscle. Within this dataset, there was no evidence that organic forms of zinc results in different levels of zinc deposition in muscle, egg or milk compared with inorganic sources. Hepatic zinc deposition levels are generally higher after supplementation with organic forms of zinc, than after supplementation with zinc oxide. This is indicative of the relatively low bioavailability of zinc oxide, as no such trend was observed when organic forms of zinc were compared with zinc sulphate. Thus, the studies provided by the applicant did not give rise to any concerns regarding different deposition patterns or bioavailabilities of inorganic and organic sources of zinc. This is consistent with previous reviews and meta-analyses of data from poultry, pigs and ruminants, which concluded that organic and inorganic sources of zinc, in general, are equivalent (Ammerman et al., 1998; Jongbloed et al., 2002; Schlegel et al., 2013; EFSA FEEDAP Panel, 2014).

3.2.2. Consumer exposure assessment

The FEEDAP Panel has summarised, in several opinions (EFSA 2012b, c, d, e; EFSA FEEDAP Panel, 2014), 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).

In all consumer groups, tissues and products of animal origin contributed to about 40–50 % of total zinc intake. Since there is no indication that the use of the different compounds of zinc under assessment would significantly modify the zinc content of tissues and products of animal origin, the exposure of consumers to zinc would also not be changed.

In its recently 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).

3.2.3. Conclusions on safety for consumers

No concerns for consumer safety are expected from the use of the zinc compounds under application in animal nutrition (zinc acetate, dihydrate; zinc chloride, anhydrous; zinc oxide; zinc sulphate, heptahydrate; zinc sulphate, monohydrate; zinc chelate of amino acids, hydrate; zinc chelate of glycine, hydrate) when used up to the maximum EU-authorised levels in feed.

3.3. Safety for the users/workers

No specific studies have been submitted for any of the compounds under assessment

3.3.1. Effects on skin, eyes and mucosae

Zinc acetate and zinc sulphate (heptahydrate and monohydrate) are irritating agents for the skin, eyes and mucosae (US NIH, 2006a, b). Zinc chloride, anhydrous, is highly hygroscopic and extremely corrosive to skin, eyes and mucosae (US NIH, 2006c).

[83] Technical Dossier/Supplementary Information (October 2012)/Annex_Qxxvi_Safety for consumer_Tissue deposition.pdf.

Zinc (metal) may cause sensitisation by skin contact; skin sensitisation to zinc sulphate, even if rarely, was reported (UKPID, 1997).

Based on the results of skin and eye irritation and skin sensitisation studies, zinc oxide is not considered an irritant for skin or eyes, or a skin sensitiser (EC, 2004).

Zinc chelate of amino acids, hydrate,[84] and zinc chelate of glycine, hydrate,[85] are skin, eye and mucosae irritants. In addition, the zinc chelate of amino acids, hydrate, may induce sensitisation by inhalation.

3.3.2. Inhalation toxicity

The FEEDAP Panel has already stated in previous opinions on zinc-based feed additives (e.g. zinc sulphate monohydrate (EFSA 2012c, d) and zinc oxide (EFSA, 2012e)), that inhalation toxicity is the main point for possible concern as regards user safety, and this is closely related to the respirable fraction in the dust of the specific zinc compounds.

The National Institute for Occupational Safety and Health (NIOSH) reported the following recommended exposure limits for zinc oxide dust: 5 mg/m^3 for a 10-hour time-weighted average, with a maximum of 15 mg/m^3 (short-term exposure limit (STEL)) (US NIOSH, 1978). The American Conference of Governmental Industrial Hygienists (ACGIH) proposed a threshold limit value (TLV) for zinc oxide of 2 mg/m^3 (zinc in respirable fraction) based on metal fume fever as the critical effect (ACGIH, 2003). The FEEDAP Panel considers it appropriate to use the ACGIH parameters for zinc oxide as the most conservative and up-to-date guidance for evaluating the risk upon inhalation exposure of the zinc compounds under assessment.

The FEEDAP Panel recognises that the use of TLV as a guidance value for the user safety of feed additives may result in overly conservative assessments, since the exposure is unlikely to be so continuous and intense as in an industrial scenario, for which TLVs have been envisaged. Indeed, the actual user exposure to dust from feed additives may be more consistent with a short-term, intermittent pattern rather than with a continuous pattern; therefore, the use of the STEL may be closer to a real-life scenario.

Nevertheless, even with the above caveat, a zinc concentration in the respirable dust exceeding the TLV by at least one order of magnitude would point out a hazard by inhalation for users.

Three compounds—zinc acetate, dihydrate; zinc chloride, anhydrous; zinc sulphate, heptahydrate—have virtually no or negligible respirable fraction; thus, they are considered to pose no appreciable risk by inhalation. However, other preparations of these additives, with different particle size distribution and dusting potential, could exist indicating that inhalatory exposure by workers cannot be excluded.

Concerning zinc oxide, a worst-case scenario can be derived from the highest values among the products of the four companies; this scenario indicates a dusting potential of 190 mg/m^3 and a fraction of up to 36 % of particles with diameter of < 10.5 µm. Thus, products with these respirable fraction and dusting potential values would lead to airborne concentrations of respirable particles of > 30-fold higher than the TLV and 6-fold higher than the STEL set by NIOSH.

Zinc sulphate, monohydrate shows a very high dusting potential (8 200 mg/m^3) and its respirable fraction is equal to 20 %. Considering the zinc content of the additive (up to 36 %), the TLV and the STEL would be exceeded, under these worst-case conditions, by two orders of magnitude and by > 50-fold, respectively.

[84] Technical Dossier/Section II/Annexes 2.5.1, 2.5.3 and 2.5.4.
[85] Technical Dossier/Section II/Annexes 2.5.8, 2.5.10 and 2.5.12.

Zinc chelate of amino acids, hydrate, and zinc chelate of glycine, hydrate, also have high dusting potentials (970 and 1 710 mg/m^3, respectively), while the respirable fractions are up to 4.5 % for the chelate of amino acids and up to 6.6 % for the chelate of glycine. Considering the zinc content of the additives (up to 17 % for the chelate of amino acids and up to 30 % for the chelate of glycine), the potential inhalation exposure to zinc from dusts of these additives corresponds, under worst-case conditions, to 7.4 mg/m^3 and 33.9 mg/m^3 for zinc chelate of amino acids, hydrate, and zinc chelate of glycine, hydrate, respectively; therefore, the potential inhalatory exposure is above the TLV but below the STEL for the chelate of amino acids, while it is one order of magnitude higher than the TLV and 3-fold higher than the STEL for the chelate of glycine. Both additives may, therefore, be a risk to users by inhalation.

3.3.3. Conclusions on safety for users/workers

Zinc acetate, zinc sulphate (heptahydrate and monohydrate), zinc chelate of amino acids, hydrate, and zinc chelate of glycine, hydrate, are irritant to the skin, eyes and mucosae. Zinc sulphate may cause skin sensitisation. Zinc chloride, anhydrous, is extremely corrosive to skin, eyes and mucosae. Zinc chelate of amino acids, hydrate, may induce sensitisation by inhalation and should be considered a skin sensitiser. Zinc oxide is not considered irritant to skin or eyes, or a skin sensitiser.

Concerning inhalation toxicity, the specific characteristics (inhalable particle fraction, dusting potential and zinc content of dust) of some of the additives assessed (zinc acetate, dihydrate; zinc chloride, anhydrous; zinc sulphate, heptahydrate) indicate that they pose no appreciable risk by inhalation. However, owing to the fact that other preparations of the additives are plausible, the FEEDAP Panel considers that all the additives under application should be treated as hazardous by inhalation.

3.4. Safety for the environment

As a consequence of the use of zinc-containing feed additives, zinc is unavoidably released into the environment. When used in livestock, zinc excreted in the faeces will enter the soil environment when faeces are applied as fertiliser to land, in the form of manure, slurry or litter. This might present two main potential risks:

- zinc accumulation within the topsoil to concentrations posing potential toxic risks to soil organisms;
- leaching of zinc from soil to surface waters in concentrations posing potential toxic risks to organisms resident in the water column and bottom sediments.

When used in aquaculture, trace elements, such as zinc, may be released directly to the broader aquatic environment around an aquaculture facility or be taken up by fish and then excreted into the environment. As stated in the EFSA Technical Guidance (EFSA, 2008b), the compartment of concern for fish farmed in cages is assumed to be the sediment, whereas for fish farmed in land-based systems the effluent flowing to surface water is considered to pose the main environmental risk.

EFSA commissioned a study on the environmental impact of zinc and copper used in animal nutrition (Monteiro et al., 2010). In this risk assessment, the Predicted No Effect Concentrations (PNECs) for the different compartments were calculated using the same methodologies as presented in the EU risk assessment report (EU-RAR) for zinc by correcting for bioavailability, based on the assumed soil and water chemistry of the different scenarios (Bodar, 2007). Likewise, it was decided to use the 'added PNEC' approach for zinc as developed in its EU-RAR (Bodar, 2007).

The emission of zinc from fish feeds (land-based and sea cages) resulted in Predicted Environmental Concentrations (PECs) below the PNECs and therefore does not give rise to concern. With regard to the terrestrial environment, the PECs in soil, simulated over a 50-year period of manure application, did not exceed the PNEC for terrestrial species in any model scenario developed. However, leaching of zinc from soil through drainage or run-off could, in some acidic soil types, potentially present an

environmental problem for surface waters. For these scenarios, the surface water PNEC will be exceeded by a factor of 3 after 10 years of the continuous application of any manure type, and up to a factor of 5 after 50 years. Predicted concentrations in the sediments of receiving waters, derived from the erosion of metal-enriched particles and transport in drainage and run-off, responded dramatically to increases in zinc inputs caused by manure application. Potential risks were predicted after ten years of continuous application for all FOCUS (Forum for the Coordination of Pesticide Fate Models and Their Use) scenarios identified in the EFSA Guidance (EFSA, 2008b) and all manure types. In most cases, the PNEC is exceeded by more than a factor of 10, especially for acidic soil types. In the view of the FEEDAP Panel, whilst worrying, these findings should be considered preliminary as further refinements of the models and their input data are feasible.

In response to the report of Monteiro et al. (2010) and the subsequent FEEDAP Opinions on zinc additives in feed under the frame of the re-evaluation (EFSA, 2012b, c, d, e), the European Commission requested EFSA to review the scope for reduced maximum content of zinc in animal feeds. This review was recently published by the FEEDAP Panel (EFSA FEEDAP Panel, 2014) and proposed, on average, a 30 % reduction in maximum authorised zinc contents in different feeds. If implemented, this would result in an overall reduction of zinc emissions from animal production by about 20 % (EFSA FEEDAP Panel, 2014). With the adoption of the newly proposed maximum zinc contents in animal feeds, the risk to the environment from the use of zinc-containing feed additives would be reduced.

3.4.1. Conclusions on safety for the environment

Based on the assessment above, the use of zinc compounds under the current application (zinc acetate, dihydrate; zinc chloride, anhydrous; zinc oxide; zinc sulphate, heptahydrate; zinc sulphate, monohydrate; zinc chelate of amino acids, hydrate; zinc chelate of glycine, hydrate) as a feed additives does not pose an immediate concern for the agricultural soil compartment. However, there is a potential environmental concern related to drainage and the run-off of zinc to surface water. Most vulnerable to these processes are acidic sandy soils. In order to draw a final conclusion, some further refinement to the assessment of zinc-based feed additives in livestock needs to be considered, for which additional data would be required. With the adoption of the newly proposed maximum zinc contents in animal feeds, the risk to the environment from the use of zinc-containing feed additives would be reduced. In addition, concerning the additives intended for use in water for drinking, the FEEDAP Panel notes that environmental safety is only valid if such use is in compliance with the EU maximum authorised zinc levels in feed.

4. Efficacy

The compounds of zinc under application for re-evaluation and their use in animal nutrition are extensively documented in scientific literature. They all are efficacious sources of zinc in meeting the animal needs, although zinc oxide is characterised by a lower bioavailability than other zinc compounds (see EFSA 2012e).

Zinc chloride, anhydrous, a new additive, is not essentially different from the currently authorised zinc chloride, monohydrate, and is considered bioequivalent to the monohydrate.

The additives under application do not require further confirmation of efficacy.

5. 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[86] and Good Manufacturing Practice.

[86] 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.

CONCLUSIONS AND RECOMMENDATIONS

The zinc compounds under consideration are: zinc acetate, dihydrate; zinc chloride, anhydrous; zinc oxide; zinc sulphate, heptahydrate; zinc sulphate, monohydrate; zinc chelate of amino acids, hydrate; zinc chelate of glycine, hydrate.

CONCLUSIONS

All the zinc compounds under application are safe sources of zinc for all animal species/categories when used up to maximum EU authorised zinc levels in complete feed. The simultaneous use of both feed and water supplemented with zinc should be avoided.

No concerns for consumer safety are expected from the use of the zinc compounds under application in animal nutrition when used up to the EU maximum authorised level in feed.

Zinc acetate, zinc sulphate (heptahydrate and monohydrate), zinc chelate of amino acids, hydrate, and zinc chelate of glycine, hydrate, are irritating agents for the skin, eyes and mucosae. Zinc sulphate may cause skin sensitisation. Zinc chloride, anhydrous, is extremely corrosive to skin, eyes and mucosae. Zinc chelate of amino acids, hydrate, may induce sensitisation by inhalation and should be considered a skin sensitiser. Zinc oxide is not considered an irritant for skin or eyes, or a skin sensitiser. The FEEDAP Panel considers that all the additives under application should be treated as hazardous by inhalation.

The use of the zinc compounds under the current application as feed additives does not pose an immediate concern for the agricultural soil compartment. However, there is a potential environmental concern related to drainage and the runoff of zinc to surface water. Most vulnerable to these processes are acidic sandy soils. In order to draw a final conclusion, some further refinements to the assessment of zinc-based feed additives in livestock need to be considered, for which additional data are required. With the adoption of the newly proposed maximum zinc contents in animal feeds, the risk to the environment from the use of zinc-containing feed additives would be reduced.

The zinc compounds under the current application are efficacious in meeting animal zinc requirements.

RECOMMENDATIONS

The Description of the additive 'Zinc chloride anhydrous' should indicate a zinc content of $\geq$ 46.1 % Zn. The compound zinc chloride, anhydrous should be only used in the form of a liquid premix.

Concerning the zinc chelate of amino acids, hydrate, the FEEDAP Panel identified, in previous assessments (EFSA, 2012b; EFSA FEEDAP Panel, 2013b) and in the current one, that the description of the compound does not meet the terms of the current legislation: 'molecular weight not exceeding 1500 Da'. Data from different companies reveal that up to 40 % of the chelates may have a molecular weight exceeding 1500 Da. The previous assessment of zinc chelate of amino acids, hydrate, considered these new findings by recommending the characterisation of the molecular weight by 'At least 90 % of the molecules should have a molecular weight not exceeding 1500 Da'. The Panel recommends to keep this definition for chelates which match this criteria. However, there would be another group of chelates 'More than 10 % of the molecules having a molecular weight exceeding 1500 Da'; consequently, this group of chelates, chemically different from the former group, requires a different name: the Panel proposes 'Chelates of protein hydrolysates'. In summary, the Panel recommends the classification of Zinc chelates into two groups, for which the following names and characteristics are proposed:

- Zinc chelate of amino acids, hydrate: not more than 10 % of the molecules exceeding 1500 Da
- Zinc chelate of protein hydrolysates: between 10 and 50 % of molecules exceeding 1500 Da

For both groups the general formula of Zn $(x)_{1-3}$•nH_2O (x= anion of any amino acid from soya protein hydrolysate) is proposed.

Measures should be taken to avoid exposure of users to the additives by inhalation and by contact with skin and eyes.

Maximum contents of total zinc in feed are set by legislation. The conclusions of the FEEDAP Panel on the safety of the seven zinc compounds assessed for the target animals, the consumer and the environment are only valid if these maximum contents are strictly adhered to during feed formulation. Since zinc is routinely supplemented to feed, only a small amount, if any, could be administered additionally via water for drinking. The exact dosing of zinc in water for drinking can only be made if the total dietary zinc content is known, which is normally not the case. Therefore, the FEEDAP Panel does not recommend the use of zinc compounds via water for drinking.

GENERAL REMARKS

A proposal to reduce the maximum currently authorised content of zinc in feed has recently been published (EFSA FEEDAP Panel, 2014). The FEEDAP Panel outlines that the conclusions on target animal safety and on consumer safety made in the current opinion need not to be modified if the reduced maximum content is put into force by legislation. However, the reduction of zinc in feed would lead to a beneficial effect on the environment; the zinc excretion from farm animals is estimated to decrease by 20 %.

The FEEDAP Panel stresses the need for analytical methods to detect organic compounds of trace elements in feed, independent from the trace element background.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier Zinc (E6). Nutritional feed additive. Compounds of trace elements. August 2010. Submitted by TREAC EEIG (Trace Elements Authorisation Consortium).

2. Dossier Zinc (E6). Nutritional feed additive. Compounds of trace elements. Supplementary information. October 2012. Submitted by TREAC EEIG (Trace Elements Authorisation Consortium).

3. Dossier Zinc (E6). Nutritional feed additive. Compounds of trace elements. Supplementary information. June 2014. Submitted by FEFANA asbl.

4. Dossier Zinc (E6). Nutritional feed additive. Compounds of trace elements. Supplementary information. January 2015. Submitted by FEFANA asbl.

5. Evaluation report of the European Union Reference Laboratory for Feed Additives on the methods(s) of analysis for Zinc (E6).

6. Comments from Member States received through the ScienceNet.

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ABBREVIATIONS

ACGIH	American Conference of Governmental Industrial Hygienists
ATSDR	Agency for Toxic Substances and Disease Registry
As	Arsenic
CAS	Chemical Abstracts Service
Cd	cadmium
CFU	colony-forming unit
CV	coefficient of variation
EC	European Commission
EU	European Union
EU-RAR	EU risk assessment report
EURL	European Union Reference Laboratory
FEFANA	EU Association of Specialty Feed Ingredients and their Mixtures
FTIR	Fourier transform infrared
Hg	mercury
ICP–AES	inductively coupled plasma atomic emission spectroscopy
MRL	maximum residue limit
NIH	National Institutes of Health
NIOSH	National Institute for Occupational Safety and Health
No	number
NRC	National Research Council
Pb	lead
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo-*para*-dioxin
PEC	predicted environmental concentration
PNEC	Predicted No Effect Concentration
SCAN	Scientific Committee on Animal Nutrition
SCF	Scientific Committee on Food
STEL	short-term exposure limit
TEQ	toxic equivalent factor
TLV	threshold limit value
TREAC EEIG	Trace Elements Authorisation Consortium European Economic Interest Group
UL	tolerable upper intake level
WHO	World Health Organization
Zn	zinc

Appendix A. Update on the biological role and toxicity of zinc

Zinc is a trace element that is essential to all known organisms, and it is the second most abundant trace element, after iron, in most vertebrates. Zinc is required for a variety of basic biological processes, including metabolism of proteins, nucleic acids, carbohydrates and lipids, and it is also involved in more complex processes, such as the immune response, neurotransmission and cell signalling (Coleman, 1992; Beyersmann, 2002; Murakami and Hirano, 2008). It has been estimated that there are approximately 3 000 zinc-containing proteins in humans (Passerini et al., 2007). Almost all of the zinc in cells is bound to proteins, peptides and amino acids, but there is a minute fluctuating pool of labile cytosolic Zn^{2+}, which is involved in cell signalling pathways (Murakami and Hirano, 2008; Haase and Rink, 2009; Hogstrand et al., 2009). One of the mechanisms by which Zn^{2+} transduces intracellular signals is by inhibition of protein tyrosine phosphatases; for example, this is believed to be the molecular mechanism behind its insulin-mimetic effect (Haase and Maret, 2003; Miranda and Dey, 2004; Wong et al., 2006). Uptake of zinc, as well as its compartmentalisation within tissues and cells, is managed principally by two large and biologically ubiquitous families of zinc transporters, the ZnT (SLC30A) family and the ZIP (SLC39A) family, which, between them, have 24 paralogues in most mammals (Feeney et al., 2005). The distinct distributions and activities of these transporters determine the distribution of zinc within cells and animals. However, cellular zinc influx may also occur via various Ca^{2+} channels and probably through some amino acid transporters.

Dietary zinc is of low toxicity to vertebrates (Clearwater et al., 2002; Van Paemel et al., 2010). Some of the most noticeable effects of zinc toxicity are impairment of copper and iron uptake, with knock-on effects on systems which depend on these metals (Eid and Ghonim, 1994; Balesaria et al., 2010). There are also effects on lipid metabolism and the immune system, as zinc is a natural regulator of functions involved in these processes. Water-breathing organisms are sensitive to waterborne zinc, with acute toxicity concentrations typically being higher than those for metals such as silver, cadmium and copper, but lower than those for manganese and nickel (McDonald and Wood, 1993). The relatively high risk of zinc toxicity to aquatic life has led to its inclusion as a 'priority pollutant' by the US Environmental Protection Agency (USEPA, 2002).

Appendix B. List of Risk Assessment Reports (RARs) on zinc and zinc compounds

In addition to the reports cited in the Background section of this Opinion, risk assessments from other EU bodies and institutions have been carried out. Bodar et al. (2005) have summarised the process of and the facts derived from the EU risk assessment of zinc and zinc compounds.

1. EU RARs

Zinc metal (CAS No 7440-66-6). Available online: http://publications.jrc.ec.europa.eu/repository/bitstream/111111111/15064/1/lbna24587enn.pdf

Zinc oxide (CAS No 1314-13-2). Available online: http://esis.jrc.ec.europa.eu/doc/existing-chemicals/risk_assessment/REPORT/zincoxidereport073.pdf

Zinc chloride (CAS No. 7646–85–7). Available online: http://esis.jrc.ec.europa.eu/doc/existing-chemicals/risk_assessment/SUMMARY/zincchlorideENVsum075.pdf

Zinc distearate (CAS No 557-05-1/91051-01-3). Available online: http://esis.jrc.ec.europa.eu/doc/existing-chemicals/risk_assessment/REPORT/zincdistearatereport074.pdf

Zinc sulphate (CAS No 7733-02-0). Available online at: http://esis.jrc.ec.europa.eu/doc/existing-chemicals/risk_assessment/SUMMARY/zincsulphateENVsum076.pdf

Trizinc bis(orthophosphate) (CAS No 7779-90-0). Available online: http://esis.jrc.ec.europa.eu/doc/existing-chemicals/risk_assessment/REPORT/zincphosphatereport077.pdf

2. EC Health and Consumers Scientific Committees Opinions

The Scientific Committee on Health and Environmental Risk (SCHER) opinion on the RARs on Zn. (http://ec.europa.eu/health/ph_risk/committees/04_scher/docs/scher_o_069.pdf)

The Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers. Opinion concerning Zinc oxide. (http://ec.europa.eu/health/ph_risk/committees/sccp/documents/out222_en.pdf)

3. EFSA-ANS Panel Opinions

Chromium picolinate, zinc picolinate and zinc picolinate dihydrate added for nutritional purposes in food supplements (http://www.efsa.europa.eu/en/efsajournal/pub/1113.htm)

Magnesium aspartate, potassium aspartate, magnesium potassium aspartate, calcium aspartate, zinc aspartate, and copper aspartate as sources for magnesium, potassium, calcium, zinc, and copper added for nutritional purposes to food supplements - Scientific Panel on Food Additives and Nutrient Sources added to food (http://www.efsa.europa.eu/en/efsajournal/pub/883.htm)

Calcium L-methionate, magnesium L-methionate and zinc mono-L-methionine sulphate added for nutritional purposes to food supplements - Scientific Opinion of the Panel on Food Additives and Nutrient Sources added to Food **(**http://www.efsa.europa.eu/en/efsajournal/pub/924.htm)

Calcium ascorbate, magnesium ascorbate and zinc ascorbate added for nutritional purposes in food supplements http://www.efsa.europa.eu/en/efsajournal/pub/994.htm

4. EFSA-AFC Panel Opinions

Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) related to calcium, magnesium and zinc malate added for nutritional purposes to food supplements as sources for calcium, magnesium and zinc and to calcium malate added for nutritional purposes to foods for particular nutritional uses and foods intended for the general population as source for Calcium (http://www.efsa.europa.eu/en/efsajournal/pub/391a.htm)

Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) related to calcium, iron, magnesium, potassium and zinc L-pidolate as sources for calcium, iron, magnesium, potassium and zinc added for nutritional purposes to food supplements and to foods intended for particular nutritional uses (http://www.efsa.europa.eu/en/efsajournal/pub/495.htm)

Magnesium L-lysinate, calcium L-lysinate, zinc L-lysinate as sources for magnesium, calcium and zinc added for nutritional purposes in food supplements—Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (http://www.efsa.europa.eu/en/efsajournal/pub/761.htm)

Opinion on certain bisglycinates as sources of copper, zinc, calcium, magnesium and glycinate nicotinate as source of chromium in foods intended for the general population (including food supplements) and foods for particular nutritional uses—Scientific Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (http://www.efsa.europa.eu/en/efsajournal/pub/718.htm)

5. EFSA-NDA Panel Opinions

Scientific Opinion on the substantiation of health claims related to zinc and maintenance of normal skin (ID 293), DNA synthesis and cell division (ID 293), contribution to normal protein synthesis (ID 293, 4293), maintenance of normal serum testosterone concentrations (ID 301), 'normal growth' (ID 303), reduction of tiredness and fatigue (ID 304), contribution to normal carbohydrate metabolism (ID 382), maintenance of normal hair (ID 412), maintenance of normal nails (ID 412) and contribution to normal macronutrient metabolism (ID 2890) pursuant to Article 13(1) of Regulation (EC) No 1924/2006 (http://www.efsa.europa.eu/en/efsajournal/pub/1819.htm)

Scientific Opinion on the substantiation of health claims related to zinc and function of the immune system (ID 291, 1757), DNA synthesis and cell division (ID 292, 1759), protection of DNA, proteins and lipids from oxidative damage (ID 294, 1758), maintenance of bone (ID 295, 1756), cognitive function (ID 296), fertility and reproduction (ID 297, 300), reproductive development (ID 298), muscle function (ID 299), metabolism of fatty acids (ID 302), maintenance of joints (ID 305), function of the heart and blood vessels (ID 306), prostate function (ID 307), thyroid function (ID 308), acid–base metabolism (ID 360), vitamin A metabolism (ID 361) and maintenance of vision (ID 361) pursuant to Article 13(1) of Regulation (EC) No 1924/2006 (http://www.efsa.europa.eu/en/efsajournal/pub/1229.htm)

Scientific Opinion on the substantiation of a health claim related to zinc and 'the prevention of bad breath by neutralising of volatile sulphur compounds in the mouth and oral cavity' pursuant to Article 13(5) of Regulation (EC) No 1924/2006 (http://www.efsa.europa.eu/en/efsajournal/pub/2169.htm)

Appendix C. List of authorisations of zinc compounds other than as feed additives

The following zinc compounds are authorised for use in food (Regulation (EC) No 1170/2009):[87] zinc acetate, zinc chloride, zinc oxide, zinc sulphate, zinc bisglycinate and zinc L-Lysinate, which may be used in the manufacture of food supplements, and zinc acetate, zinc chloride, zinc oxide, zinc sulphate and zinc bisglycinate, which may be added to food. Zinc acetate (E-650) is authorised as a food additive for its use in chewing gum at a maximum level of 1 000 mg/kg (European Parliament and Council Directive No 95/2/EC).[88]

The following zinc compounds can be used for the manufacture of dietetic foods (Commission Regulation (EC) No 953/2009):[89] zinc acetate, zinc chloride, zinc citrate, zinc gluconate, zinc lactate, zinc oxide, zinc carbonate, zinc sulphate and zinc bisglycinate.

The following zinc compounds can be used for the manufacturing of processed cereal-based foods and foods for infants and young children (Commission Directive 2006/125/EC):[90] zinc, zinc acetate, zinc citrate, zinc lactate, zinc sulphate, zinc oxide and zinc gluconate.

The following zinc compounds are listed in Table 1 of the Annex of Regulation 37/2010[91] as 'Allowed substances, no MRL required': zinc acetate, zinc aspartate, zinc chloride, zinc gluconate, zinc oleate, zinc oxide, zinc stearate and zinc sulphate.

The following zinc compound is listed in Annex of Commission Implementing Regulation (EU) No 540/2011[92] as 'Active substances approved for use in plant protection products': Trizinc diphosphide (zinc phosphide).

The following types of fertilisers containing zinc and described as 'Fertilisers containing only one micro-nutrient' are listed in Annex I of Regulation (EC) No 2003/2003[93] of the European Parliament and of the Council as: (a) zinc salt (chemically obtained product and having as its essential ingredient a mineral salt of zinc), (b) zinc chelate (water-soluble product obtained by combining zinc chemically with a chelating agent), (c) zinc oxide (chemically obtained product and having as its essential ingredient zinc oxide), (d) zinc-based fertiliser (product obtained by mixing types 'a' and 'c'), zinc-based fertiliser solution (product obtained by dissolving types 'a' and/or one of type 'b' in water).

The following zinc compounds can be used for cosmetic purposes (Regulation (EC) No 1223/2009 of the European Parliament and of the Council[94]): zinc acetate, zinc chloride, zinc gluconate, zinc glutamate, zinc phenolsulfonate, zinc oxide, zinc stearate, zinc pyrithione and zinc peroxide.According to the Annex to Regulation (EC) No 432/2012,[95] the following health claims can be made only for

[87] 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.

[88] European Parliament and Council Directive No 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners. OJ L 61, 18.3.1995, p. 1.

[89] 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.

[90] Commission Directive 2006/125/EC of 5 December 2006 on processed cereal-based foods and baby foods for infants and young children. OJ L 339, 6.12.2006, p. 16.

[91] Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. OJ L 15, 20.1.2010, p. 1.

[92] Commission Implementing Regulation (EU) No 540/2011 of 25 May 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the list of approved active substances. OJ L 153, 11.6.2011, p. 1.

[93] 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.

[94] Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products. OJ L 342, 22.12.2009, p. 59.

[95] 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, 25.05.2012, p.1.

food which is at least a source of zinc, 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:[96] zinc contributes to normal acid-base metabolism, zinc contributes to normal carbohydrate metabolism, zinc contributes to normal cognitive function, zinc contributes to normal DNA synthesis, zinc contributes to normal fertility and reproduction, zinc contributes to normal to macronutrient metabolism, zinc contributes to normal metabolism of fatty acids, zinc contributes to normal metabolism of vitamin A, zinc contributes to normal protein synthesis, zinc contributes to the maintenance of normal bones, zinc contributes to the maintenance of normal hair, zinc contributes to the maintenance of normal nails, zinc contributes to the maintenance of normal skin, zinc contributes to the maintenance of normal testosterone levels on the blood, zinc contributes to the maintenance of normal vision, zinc contributes to the normal function of the immune system, zinc contributes to the protection of cells from oxidative stress, zinc has a role in the process of cell division.

[96] 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.

Appendix D. Details on the characterisation and identity of the compounds for which more than one company is involved in the application[97]

Table 4: Parameters of characterisation and identity of zinc oxide (number of batches in brackets)

	Company[(A)]			
	d2	**d5**	**d7**	**d14**
Zinc content (%)	75-77 (5)[98]	72-75 (4)[99]	79-80 (5)[100]	72-73 (5)[101]
Pb (mg/kg)	74-180 (8)[102]	73-103 (4)[103]	7-18 (5)[104]	224-343 (5)[105]
Cd (mg/kg)	4-12 (8)	<0.1-0.3 (3)	0.9-2.7 (5)	8-11 (5)
Hg (mg/kg)	---	---	< 0.10 (5)	Not Detected (3)
As (mg/kg)	19-29 (8)	29-56 (4)	0.6-1.1 (5)	28-61 (5)
Dioxins[(B)]	0.079 (1)[106]	0.040-0.050 (6)[107]	0.19-0.20 (5)[108]	0.18-0.19 (2)[109]
Dioxins-like-PCBs[(C)]	0.039 (1)			
Sum of dioxins and dioxin-like PCBs[(D)]	---	0.086 (1)	0.22-0.28 (5)	0.21-0.22 (2)
Particle size (%)[(E)]	61.3 (< 50 µm) 9.33 (< 10 µm) (1)[110]	16.6 (< 52.2 µm) 0.83 (< 11 µm) (3)[111]	90.8 (< 45 µm) --- (< 10 µm) (5)[112]	94.8 (< 51 µm) 36.3 (< 10.5 µm) (1)[113]
Dusting potential (g/m^3)	---	---	---	0.19 (1)[114]

(A) Four companies: d2, d5, d7 and d14.
(B) Expressed as ng WHO-PCDD/F-TEQ/kg.
(C) Expressed as ng WHO-PCBs-TEQ/kg.
(D) Expressed as ng WHO-PCDD/F- PCB-TEQ/kg.
(E) Technique used. Laser diffraction: d2, d5 and d14 (expressed as v/v); sieving: d7 (expressed as w/w).
--- Not provided.

97 This section has been amended following the confidentiality claims made by the applicant.
98 Technical Dossier/Section II/Annex 2.1.26.
99 Technical Dossier/Section II/Annex 2.1.27.
100 Technical Dossier/Section II/Annex 2.1.28.
101 Technical Dossier/Section II/Annex 2.1.30.
102 Technical Dossier/Section II/Annex 2.1.47 (for Pb, Cd and As).
103 Technical Dossier/Section II/Annex 2.1.48 and Technical Dossier/Supplementary Information (October 2012)/Annex_Qv_Zn oxide_dioxins and PCBs_Company (d5).pdf (for Pb, Cd and As).
104 Technical Dossier/Section II/Annex 2.1.49 (for Pb, Cd, Hg and As).
105 Technical Dossier/Section II/Annex 2.1.51 (for Pb, Cd, Hg and As).
106 Technical Dossier/Section II/Annex 2.1.69.
107 Technical Dossier/Section II/Annex 2.1.70 and Technical Dossier/Supplementary Information (October 2012)/ Annex_Qv_Zn oxide_dioxins and PCBs_Company (d5).pdf..
108 Technical Dossier/Section II/Annex 2.1.71.
109 Technical Dossier/Section II/Annex 2.1.73.
110 Technical Dossier/Section II/Annex 2.2.34.
111 Technical Dossier/Section II/Annex 2.2.35.
112 Technical Dossier/Section II/Annex 2.2.36.
113 Technical Dossier/Section II/Annex 2.2.38.
114 Technical Dossier/Supplementary Information (October 2012)/Annex_Qviii_Zn oxide_Dusting potential_Company (d14).pdf.

Table 5: Parameters of characterisation and identity of zinc chelate of amino acid, hydrate (number of batches in brackets)

	Company[A]				
	d1	**d2**	**d4**	**d8**	**d13**
Zinc content (%)	12.3-12.5 (3)[115]	15.2-16.1 (5)[116]	15.5-16.6 (5)[117]	14-15.2 (3)[118]	15.1-16.1 (5)[119]
Pb (mg/kg)	<0.1-0.81 (3)[120]	7-24 (9)[121]	<0.50 (3)[122]	<0.5 (3)[123]	<0.1-0.30 (3)[124]
Cd (mg/kg)	1.6-4.5 (3)	1-5 (9)	<0.50-2.52 (3)	0.5-2.0 (3)	0.42-1.7 (3)
Hg (mg/kg)	<0.005 (3)	---	<0.02 (3)	<0.5 (3)	<0.01-0.05 (3)
As (mg/kg)	<0.2-0.3 (3)	<1 (1)	<0.50 (3)	1.0-2.3	<0.10-0.2 (3)
F (mg/kg)	48-60 (3)	---	---	---	---
Dioxins[B]	0.046-0.051 (3)[125]	0.12-0.15 (2)[126,127]	0.09 (3)[128]	0.004-0.19 (4)[129]	0.09 (6)[130]
Sum of dioxins and dioxin-like PCBs[C]	0.070-0.079 (3)	0.19 (1)	0.124-0.150 (3)	0.004-0.21 (4)	0.125 (3)[131]
Particle size (%)[D]	70.1 (<45.4 µm) 4.48 (<10 µm) (4)[132]	20.8 (<50 µm) 3.73 (<10 µm) (1)[133]	4.3 (<63 µm) (3)[134]	3.14 (<50 µm) 0.0 (<10 µm) (3)[135]	57.2 (<44 µm) 3.24 (<11 µm) (1)[136]

115 Technical Dossier/Section II/Annex 2.1.13.
116 Technical Dossier/Section II/Annex 2.1.14.
117 Technical Dossier/Section II/Annex 2.1.16.
118 Technical Dossier/Section II/Annex 2.1.18.
119 Technical Dossier/Section II/Annex 2.1.19
120 Technical Dossier/Section II/Annex 2.1.34 (for heavy metals and Arsenic content).
121 Technical Dossier/Section II/Annex 2.1.35 (for heavy metals and Arsenic content).
122 Technical Dossier/Section II/Annex 2.1.37 (for heavy metals and Arsenic content).
123 Technical Dossier/Section II/Annex 2.1.39 (for heavy metals and Arsenic content).
124 Technical Dossier/Section II/Annex 2.1.40 (for heavy metals and Arsenic content).
125 Technical Dossier/Section II/Annex 2.1.56 (for dioxins and dioxins+dioxin-like PCBs).
126 Technical Dossier/Section II/Annex 2.1.57.
127 Technical Dossier/Supplementary Information (October 2012)/Annex_Qxvi_Zn AA_Dioxins and PCBs_Company (d2).pdf.
128 Technical Dossier/Section II/Annex 2.1.59 (for dioxins and dioxins+dioxins like PCBs).
129 Technical Dossier/Section II/Annex 2.1.61 (for dioxins, dioxin-like PCBs and dioxins+dioxin-like PCBs).
130 Technical Dossier/Section II/Annex 2.1.62. Technical Dossier/Supplementary Information (October 2012)/Annex_Qxvi_Zn AA_Dioxins and PCBs_TN.pdf.pdf (for dioxins and dioxin-like PCBs).
131 Technical Dossier/Supplementary Information (October 2012)/Annex_Qxvi_Zn AA_Dioxins and PCBs_TN.pdf.pdf (for dioxins and dioxin-like PCBs).
132 Technical Dossier/Section II/Annex 2.2.21.
133 Technical Dossier/Section II/Annex 2.2.22.
134 Technical Dossier/Section II/Annex 2.2.24.
135 Technical Dossier/Section II/Annex 2.2.26.

	Company[A]				
	d1	**d2**	**d4**	**d8**	**d13**
Dusting potential (g/m^3)	0.97 (4)[137]	---	---	---	---

(A) Five companies: d1, d2, d4, d8 and d13
(B) Expressed as ng WHO-PCDD/F-TEQ/kg.
(C) Expressed as ng WHO-PCDD/F- PCB-TEQ/kg.
(D) Technique used. Laser diffraction: d1, d2, d8 and d3 (expressed as v/v); sieving: d4 (expressed as w/w).
--- Not provided.

[136] Technical Dossier/Section II/Annex 2.2.27.
[137] Technical Dossier/Supplementary Information (October 2012)/Annex_Qxviii_Zn chelate AA_Dusting potential_Company (d1).pdf.

Table 6: Microbiological contaminants of representative batches of zinc chelate of amino acids, hydrate

Company [(A)] (Number of batches)	Production date Date of analysis	Age (months)	Contaminants	CFU/g
d1 (1)[138]	March 2009	36	Total aerobic plate count	$<1.0 \times 10^2$
			Total Yeasts and Moulds	$<1.0 \times 10^1$
	April 2012		Coliforms	$<1.0 \times 10^2$
			Salmonella	Negative in 25 g
d2 (1)[139]	November 2009	ca. 26	Total plate count	$<1.0 \times 10^1$
			Total Yeasts and Moulds	Negative in 0.1g
	January 2012		*E. coli*	$<1.0 \times 10^1$
			Salmonella	Negative in 25 g
d4 (1)[140]	April 2009	31	Yeasts	$<1.0 \times 10^2$
			Moulds	$<1.0 \times 10^2$
	December 2011		*Salmonella*	Not detected in 25g
			Total aerobic count	$<1.0 \times 10^2$
			E. coli	$<1.0 \times 10^1$
			Coliform bacteria	$<1.0 \times 10^2$
			Bacillus cereus	$<1.0 \times 10^2$
d8 (1)[141]	October 2008	ca. 38	Yeasts	$<2.0 \times 10^1$
			Moulds	$<2.0 \times 10^1$
	December 2011		*Salmonella*	Negative in 25 g
			Total bacterial count - aerobic	$<0.5 \times 10^1$
			Clostridium perfringens	$<0.5 \times 10^1$
			Clostridium botulinum	Negative in 25 g
d13 (2)[142]	Oct./Nov. 2011	ca.3	Yeasts	$<1.0 \times 10^2$
			Moulds	$<1.0 \times 10^2$
	Jan./Feb. 2012		*Salmonella*	Negative in 25g
			Total bacterial count - aerobic	$<1.0 \times 10^2$
			Total Coliforms	$<1.0 \times 10^1$
(3)[143]	Aug./Sept. 2008	ca. 36	Yeasts	$<1.0 \times 10^2$
			Moulds	$<1.0 \times 10^2$
	Jan./Feb. 2012		*Salmonella*	Negative in 25g
			Total plate count - aerobic	$<1.0 \times 10^2$
			Total Coliforms	$<1.0 \times 10^1$

(A) Five companies: d1, d2, d4, d8 and d13.

[138] Technical Dossier. FAD-2010-0142_SIn_Oct12. Annexes. Annex_Qxvii_Zn chelate AA_mycotoxins and microbio_Company (d1).pdf.
[139] Technical Dossier. FAD-2010-0142_SIn_Oct12. Annexes. Annex_Qxix_Zn chelate AA_microbio_Company (d2).pdf.
[140] Technical Dossier. FAD-2010-0142_SIn_Oct12. Annexes. Annex_Qxix_Zn chelate AA_microbio_Company (d4).pdf.
[141] Technical Dossier. FAD-2010-0142_SIn_Oct12. Annexes. Annex_Qxvii_Zn chelate AA_mycotoxins and microbio_Company (d8).pdf.
[142] Technical Dossier. FAD-2010-0142_SIn_Oct12. Annexes. Annex_Qxvii_Zn chelate AA_mycotoxins and microbio_TN.pdf.
[143] Technical Dossier. Company (d13)_Zinc chelate AA hydrate_Product shelf life_2008_Sect II_08.12.12.pdf.

Table 7: Main characteristics of zinc chelate of glycine, hydrate (powder), as supplied by four companies (number of batches in brackets)

	Company[A]				
	d2		d4	d8	d12
	Higher grade	Lower grade[144]			
Zinc Content (%)	25.1-25.8 (5)[145]	15.6-16.0 (5)	26.0-26.6 (5)[146]	26.8 (1)[147]	30.3-30.9 (5)[148]
Pb (mg/kg)	4-22 (7)[149]	20-28 (5)	<0.5 (3)[150]	<10 (4)[151]	<1-1.31 (3)[152]
Cd (mg/kg)	1.5-4 (7)	2-4 (5)	0.79-2.66 (3)	<1 (4)	<1 (3)
Hg (mg/kg)	---	---	<0.02 (3)	<1 (4)	<0.0007-<0.0024 (3)
As (mg/kg)	1 (1)	1-8 (5)	<0.5 (3)	<1 (4)	<0.01 (3)
Dioxins[B]	0.17 (1)[153]	0.049-0.098 (5)	0.009 (2)[154]	0.17 (1)[155]	0.027 (1)[156]
Dioxin-like-PCBs[C]	0.0021	0.01-0.071 (5)		0.06 (1)	
Sum of Dioxins and Dioxin-like-PCBs[D]	---	---	0.128-0.138 (2)	---	0.028 (1)
Particle size (%)[E]	44.6 (< 50 µm) 6.59 (< 10 µm) (1)[157]	---	0.33 (< 52.6 µm) 0.16 (< 10 µm) (1)[158]	1.29 (< 50 µm) 0.0 (< 10 µm) (3)[159]	25 (< 56 µm) 3.0 (< 10 µm) (1)[160]
Dusting potential (g/m^3)	1.71[161]	---	---	---	---

[144] Technical Dossier/Supplementary Information (October 2012)/Annex_New data_COMPANY (D2)_Composition_Zinc chelate glycine_15%_5CoAs.pdf.
[145] Technical Dossier/Section II/Annex 2.1.20.
[146] Technical Dossier/Section II/Annex 2.1.21.
[147] Technical Dossier/Section II/Annex 2.1.22a.
[148] Technical Dossier/Section II/Annex 2.1.23.
[149] Technical Dossier/Section II/Annex 2.1.41 (for heavy metals and Arsenic content).
[150] Technical Dossier/Section II/Annex 2.1.42 (for heavy metals and Arsenic content).
[151] Technical Dossier/Section II/Annex 2.1.43 (for heavy metals and Arsenic content).
[152] Technical Dossier/Section II/Annex 2.1.44 (for heavy metals and Arsenic content).
[153] Technical Dossier/Section II/Annex 2.1.63.
[154] Technical Dossier/Section II/Annex 2.1.64.
[155] Technical Dossier/Section II/Annex 2.1.65.
[156] Technical Dossier/Section II/Annex 2.1.66.
[157] Technical Dossier/Section II/Annex 2.2.28.
[158] Technical Dossier/Section II/Annex 2.2.29.
[159] Technical Dossier/Section II/Annex 2.2.30.
[160] Technical Dossier/Section II/Annex 2.2.31.
[161] Technical Dossier/Supplementary Information (October 2012)/Annex_Qxx_Zn glycinate_Dusting potential_Company (d2).pdf.

(A) Four companies: d2, d4, d8 and d12
(B) Expressed as ng WHO-PCDD/F-TEQ/kg.
(C) Expressed as ng WHO-PCBs-TEQ/kg.
(D) Expressed as ng WHO-PCDD/F-PCB-TEQ/kg.
(E) Technique used. Laser diffraction (expressed v/v) .
--- Not provided

Table 8: Composition of the zinc chelate of glycine, hydrate (powder), as supplied by four companies (number of batches in brackets)

	Company[(A)]			
	d2-Lower grade[162]	**d4**[163]	**d8**[164]	**d12**[165]
Zinc Content (%)	15.8-16.3 (3)	24.8-25.1 (3)	27.0-27.3 (3)	25.7 (1)
Extractable glycine (%)	52.2-52.6 (3)	33.3-35.1 (3)	29.9-30.4 (3)	64.3 (1)
Sulphur (%)	8.4-8.8 (3)	12.8-13.0 (3)	13.5-13.8 (3)	0.1 (1)
Sulphate (%)	25.1-26.5 (3)	36.0-38.7 (3)	40.7-41.3 (3)	0.3 (1)
Moisture (%)	5.1-5.9 (3)	1.4-2.7 (3)	1.1-1.9 (3)	9.6 (1)

(A) Four companies: d2, d4, d8 and d12

[162] Technical Dossier/ Supplementary Information (January 2015)/ ZnGly_Composition_Company (d2).pdf.
[163] Technical Dossier/ Supplementary Information (January 2015)/ ZnGly_Composition_Company (d4).pdf.
[164] Technical Dossier/ Supplementary Information (January 2015)/ ZnGly_Composition_Company (d8).pdf.
[165] Technical Dossier/ Supplementary Information (January 2015)/ ZnGly_Composition_Company (d12).pdf.

Appendix E. Zinc deposition in liver and muscle

Table 9: Summary of the studies provided by the applicant concerning zinc deposition in liver and muscle resulting from organic or inorganic zinc supplementation to animals

Animal	Zinc (mg/kg feed)		Deposition in liver (mg Zn/kg liver)		Deposition in muscle (mg Zn/kg muscle)		Reference
	Total (analysed zinc)	Supplemented	Inorganic	Organic	Inorganic	Organic	
Broiler ZnO/ Zn-Gly	75	n.d.	26[a]	29[b]	19[a]	21[b]	Tronina et al., 2011
Piglet $ZnSO_4$/ Zn-Lys	2950	3000	1883[a]*	1765[b]*	105[a]*	99[b]*	Schell and Kornegay, 1996
Piglet $ZnSO_4$/ Zn-Lys	2190	2000	949[a]*	654[b]*	n.d.	n.d	Schell and Kornegay, 1996
Piglet $ZnSO_4$/ Zn-Lys	960	1000	156[a]*	130[b]*	n.d.	n.d	Schell and Kornegay, 1996
Piglet $ZnSO_4$/ Zn-Prot	130	100	563*	642*	n.d	n.d.	Payne et al., 2006
Dairy calf ZnO/ Zn-AA (Lys, Met)	370	300	259[a]	387[b]	n.d.	n.d	Kincaid et al., 1997
Dairy calf $ZnSO_4$ / Zn-Prot	n.d.	20	157[a]*	133[b]*	n.d.	n.d.	Wright and Spears, 2004
Beef steer $ZnSO_4$/ Zn-Gly	43	20	90[a]	114[b]	n.d.	n.d.	Spears et al., 2004
Sheep $ZnSO_4$ / Zn-Lys	446	360	195[a]*	389[b]*	261*	267*	Rojas et al., 1995
Sheep $ZnSO_4$ / Zn-Met	421	360	195*	198*	261*	259*	
Rainbow trout Zn-Prot/ $ZnSO_4$	275	n.d.	18	19	5	5	Rider et al., 2008
Sea bass ZnO/ Zn-Prot	250	150	57[a]	67[b]	21[a]	16[b]	Fountoulaki et al., 2010

*: Dry matter basis.

a-b: within 'Deposition in liver' or 'Deposition in muscle', values with different letter superscripts are significantly different ($P \leq 0.05$).

AA, amino acid; Gly, glycine; Lys, lysine; Met, methionine; n.d., not described; Prot, protein or hydrolysed protein.

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 (E6)[1]

In the current application authorisation is sought under articles 4(1) and 10(2) for *zinc acetate dihydrate*[2], *zinc chloride anhydrous*[2], *zinc oxide*[2,3], *zinc sulphate heptahydrate*[2], *zinc sulphate monohydrate*[2,4,5], *zinc chelate of amino acids hydrate*[2,6] and *zinc chelate of glycine hydrate*[2] under the category/functional group 3(b) of 'nutritional additives'/'compounds of trace elements', according to the classification system of Annex I of Regulation (EC) No 1831/2003.

According to the Applicants: - *zinc acetate dihydrate* is white solid with a minimum content of 29.6 % *total zinc*; - *zinc chloride anhydrous* is white to slightly coloured solid with a minimum content of 46 % *total zinc*; - *zinc oxide* is white to dark green or beige brownish solid with a minimum content of 72 % *total zinc*; - *zinc sulphate heptahydrate* is a white solid with a minimum content of 22 % *total zinc;* - *zinc sulphate monohydrate* is a white to cream coloured solid with a minimum content of 34 % *total zinc*; - *zinc chelate of amino acid hydrate* is beige to dark tanned solid with a minimum content of 10 % *total zinc*; and - *zinc chelate of glycine hydrate* is white to cream coloured solid with a minimum content of 24 % *total zinc*. Specifically, authorisation is sought for the use of these *feed additives* for all categories and species.

For the identification and quantification of the inorganic zinc compounds (i.e. *zinc acetate dihydrate, zinc chloride anhydrous, zinc oxide, zinc sulphate heptahydrate and zinc sulphate monohydrate*) in the *feed additive*, the EURL recommends for official control the relevant European Pharmacopoeia Monograph (1482, 0110, 0252, 0111 and 2159) methods, based on complexometric titration with 0.1 M sodium EDTA using xylenol orange triturate as indicator.

For the quantification of 'amino' content in the amino zinc chelates (i.e. z*inc chelate of glycine hydrate* and *zinc chelate amino acids hydrate*), the Applicant proposed - upon request from the EURL - the Community method based on High Performance Liquid Chromatography (HPLC) combined with post-column derivatisation using ninhydrin as derivatisation agent and photometric detection at 570 nm. The EURL considers the Community method suitable for the characterisation of the amino compounds in the frame of official control.

For the *determination* of total zinc in all the *feed additives*, *premixtures* and *feedingstuffs* the Applicant submitted the CEN method (EN 15510), based on inductively coupled plasma atomic emission spectroscopy (ICP-AES). The following performance characteristics were reported: - a relative standard deviation of *repeatability* (RSD_r) ranging from 1.7 to 8.8 %; - a relative standard deviation for *reproducibility* (RSD_R) ranging from 5.0 to 19 %; and - a limit of quantification (LOQ) of 3 mg/kg. Furthermore, the Applicant identified an alternative CEN ring-trial validated method (CEN/TS 15621) based on ICP-AES after pressure digestion, for the determination of total zinc in the *feed additive*, *premixtures* and *feedingstuffs*. The following performance characteristics were reported for a feed for pigs, and for sheep, a rock phosphate, a mineral premix and a mineral mix, where the total zinc content ranged from 26.6 to 3618 mg/kg: - RSD_r ranging from 1.5 to 5.4 %; - RSD_R ranging from 2.7 to 22 %; and - LOQ = 1 mg/kg *feedingstuffs*. Finally, the Applicant suggested the Community method for the determination of total zinc in *feedingstuffs*, with limited method performance characteristics provided. However, the UK Food Standards Agency organised a comparative trial based on the above mentioned Community method and reported precisions (RSD_r and RSD_R) for *feedingstuffs* ranging from 1.0 to 9.5 %. Based on these acceptable method performance characteristics the EURL recommends for official control the ICP-AES CEN methods (EN 15510 and CEN/TS 15621) to determine total zinc content by in the *feed additive* and *premixtures*. As for the determination of total zinc content in

[1] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/default/files/FinRep-SANCO-Zinc.pdf
[2] FAD-2010-0142.
[3] FAD-2010-0072.
[4] FAD-2010-0228.
[5] FAD-2010-0059.
[6] FAD-2010-0063.

feedingstuffs, the EURL recommends for official control the Community method based on AAS together with the above mentioned ICP-AES CEN methods.

For the quantification of total zinc in *water* the Applicant[2] submitted the ring trial validated method EN ISO 11885, based on ICP-AES. The following performance characteristics are reported: - RSD_r ranging from 1.5 to 2.4 %; - RSD_R ranging from 4.9 to 5.9 %; and LOQ = 1 µg/L. Based on these acceptable method performance characteristics the EURL recommends for official control the CEN methods (EN ISO 11885) to quantify total zinc content by ICP-AES in the *water*.

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.