SCIENTIFIC OPINION

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Safety and efficacy of manganese compounds (E5) as feed additives for all animal species: manganous carbonate; manganous chloride, tetrahydrate; manganous oxide; manganous sulphate, monohydrate; manganese chelate of amino acids, hydrate; manganese chelate of glycine, hydrate, based on a dossier submitted by FEFANA asbl

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)

Abstract

The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) has assessed five manganese compounds: manganous chloride, tetrahydrate; manganous sulphate, monohydrate; manganous oxide; manganese chelate of amino acids, hydrate; manganese chelate of glycine, hydrate. The safety assessment is based on the assumption that the current maximum total contents of manganese authorised in feed are respected. All manganese compounds under application are considered safe for all animal species/categories. The mean manganese intake of the European population includes already the manganese from animal products and does not pose a toxicological concern. The effect of dietary manganese fed to animals on tissue concentrations is limited. Supplementation of feed with the manganese compounds under assessment would consequently not affect consumer exposure and is of no concern for consumer safety. All manganese compounds are considered as eye irritants, the manganese chelate of glycine and the manganese chelate of amino acids as irritants to skin and the latter one as dermal sensitiser. However, the presence of nickel in all additives may induce contact dermatitis. Exposure to manganese in dust of all additives and to nickel (except manganous chloride and manganous oxide) poses a risk to users by inhalation. The use of the manganese compounds under assessment in animal nutrition for all animal species is not expected to pose a risk to the environment. The manganese compounds under assessment are recognised as efficacious sources of manganese in meeting animals' requirements.

Keywords: nutritional additive, compounds of trace elements, manganese, manganese compounds, safety, environment, efficacy

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Summary

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on safety and efficacy of manganese compounds as feed additives for all animal species: manganous chloride, tetrahydrate; manganous sulphate, monohydrate; manganous oxide; manganese chelate of amino acids, hydrate; manganese chelate of glycine, hydrate. The safety assessment is based on the assumption that the current maximum total contents of manganese authorised in feed are respected.

All manganese compounds under application are considered safe for all animal species/categories.

The mean manganese intake of the European population includes already the manganese from animal products and does not pose a toxicological concern. The effect of dietary manganese fed to animals on tissue concentrations is limited. Supplementation of feed with the manganese compounds under assessment would consequently not affect consumer exposure and is of no concern for consumer safety.

All manganese compounds are considered as eye irritants, the manganese chelate of glycine and the manganese chelate of amino acids as irritants to skin and the latter one as dermal sensitiser. However, the presence of nickel in all additives may induce contact dermatitis. Exposure to manganese in dust of all additives and to nickel (except manganous chloride and manganous oxide) poses a risk to users by inhalation.

The use of the manganese compounds under assessment in animal nutrition for all animal species is not expected to pose a risk to the environment.

The manganese compounds under assessment are recognised as efficacious sources of manganese in meeting animals' requirements.

The FEEDAP Panel made some recommendations concerning (i) the monitoring of mercury and nickel in the additives; (ii) the characterisation of manganese chelates of amino acids, hydrate; (iii) the protection of the user; and (iv) the use of manganese-based additives in water for drinking, which is seen as critical to ensure compliance with the legally established maximum supply of manganese to animals.

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1. Introduction

1.1. Background and Terms of Reference[1]

Regulation (EC) No 1831/2003[2] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7. 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 1 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 7 years after the entry into force of this Regulation for additives authorised without a time limit or pursuant to Directive 82/471/EEC.

The European Commission (EC) received a request from TREAC EEIG (Trace Elements Authorisation Consortium European Economic Interest Grouping)[3,4] for (i) authorisation of a new use and/or (ii) re-evaluation of authorisation, of manganese-containing additives 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) as an application 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 dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 30 May 2012.

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. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the product 'Manganous chloride, tetrahydrate', 'Manganous sulphate, monohydrate', 'Manganous oxide'; 'Manganese chelate of amino acids, hydrate' and 'Manganese chelate of glycine, when used under the proposed conditions of use (see Section 3.1.7).[5]

1.2. Additional information

Manganese (Mn) is an abundant element which makes up about 0.1% of the Earth's crust. It exists in a variety of oxidation states, Mn^{2+} and Mn^{3+} being the most biologically important. The elemental (metal) form of manganese does not occur naturally in the environment; however, manganese is a component of over 100 minerals (ATSDR, 2012).

Manganese is an essential nutrient in humans and animals that plays a role in bone mineralisation, regulation of protein and energy metabolism, cellular protection from damaging free radical species and formation of glycosaminoglycans (Wedler, 1994). Manganese acts as both (i) a constituent of metalloenzymes, e.g. arginase, pyruvate carboxylase and manganese-superoxide dismutase (NRC, 1989; Keen and Zidenberg-Cher, 1990; Wedler, 1994) and (ii) as activator of enzymes involved with either a catalytic or regulatory function (e.g. transferases, decarboxylases, hydrolases) (Wedler, 1994). In its activating capacity, manganese can bind either to a substrate (such as adenosine triphosphate) or directly to a protein, thereby causing conformational changes (Keen and Zidenberg-Cher, 1990).

[1] This section has been amended following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.
[2] 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.
[3] TREAC EEIG – Trace Elements Authorisation Consortium. Avenue Louise 120, Box 13. 1050-Brussels. Belgium. This application involves 12 companies, which have been nominated in the text as (b1) to (b12).
[4] On 19/12/2012, the rights of TREAC EEIG were transferred to FEFANA asbl, Avenue Louise 130A-Box 1, 1050 Brussels, Belgium.
[5] The compound 'Manganous carbonate' was withdrawn from the application during the assessment.

The additives 'Manganous chloride, tetrahydrate', 'Manganous sulphate, monohydrate', 'Manganous oxide', 'Manganese chelate of amino acids, hydrate' and 'Manganese chelate of glycine, hydrate' had been authorised in the European Union (EU) under the element Manganese-Mn (E5) for all animal species 'Without a time limit' (Commission Regulation (EC) No 1334/2003[6] and Commission Regulation (EC) No 479/2006)[7] 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'.[8]

The Scientific Committee on Animal Nutrition (SCAN) issued a report on the use of manganomanganic oxide in feedingstuffs (EC, 2002). EFSA issued an opinion on the safety of the chelated forms of iron, copper, manganese and zinc with synthetic feed grade glycine (EFSA, 2005) and three opinions on a manganese chelate of hydroxy analogue of methionine (EFSA, 2008a, 2009a, 2010a). In the frame of re-evaluation EFSA has delivered three opinions on manganese-based additives: manganese chelate of amino acids, hydrate (EFSA FEEDAP Panel, 2013a), manganous oxide (EFSA FEEDAP Panel, 2013b, 2013c) and manganous sulphate monohydrate (EFSA FEEDAP Panel, 2013c).

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

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[9] in support of the authorisation request for the use of five manganese compounds (manganous chloride, tetrahydrate; manganous oxide; manganous sulphate, monohydrate; manganese chelate of amino acids, hydrate; manganese chelate of glycine, hydrate) as feed additives. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008 and the applicable EFSA guidance documents.

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

EFSA commissioned the University of Gent (Belgium) to carry out a study on the biological role, content in feed and requirements in animal nutrition of selected trace and ultratrace elements, including manganese. The findings were submitted to EFSA in the form of a technical report (Van Paemel et al., 2010). Information from this report has been used in this opinion.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of manganese (five compounds, including: manganous chloride, tetrahydrate; manganous oxide; manganous sulphate, monohydrate; manganese chelate of amino acids, hydrate; manganese chelate of glycine, hydrate) in animal feed. The Executive Summary of the EURL report can be found in the Annex.[10]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and efficacy of five manganese compounds (manganous chloride, tetrahydrate; manganous oxide; manganous sulphate, monohydrate; manganese chelate of amino acids, hydrate; manganese chelate of glycine, hydrate) is

[6] 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.
[7] 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.
[8] European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003. http://ec.europa.eu/food/food/animalnutrition/feedadditives/comm_register_feed_additives_1831-03.pdf
[9] FEED dossier reference: FAD-2010-0088.
[10] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/default/files/FinRep-SANCO-Manganese.pdf

in line with the principles laid down in Regulation (EC) No 429/2008[11] and the relevant guidance documents: Guidance on nutritional additives (EFSA FEEDAP Panel, 2012a), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008b), Guidance for the preparation of dossiers for the re-evaluation of certain additives already authorised under Directive 70/524/EEC (EFSA, 2008c), Guidance for the preparation of dossiers for additives already authorised for use in food (EFSA FEEDAP Panel, 2012b), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012c), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012d).

3. Assessment

The opinion is based on data provided by the companies joint in the application Consortium[12] involved in the production/distribution of manganese-containing compounds, and publicly available literature. It should be recognised that these data cover only a fraction of the existing manganese-based additives placed on the market.

The additives under assessment are: manganous chloride, tetrahydrate; manganous oxide; manganous sulphate, monohydrate; manganese chelate of amino acids, hydrate; manganese chelate of glycine, hydrate. These compounds are already authorised in the EU for use in feed. Additionally, some of the compounds are applied for use in water. Therefore, a re-evaluation of authorisation, or a new use of an additive is sought, depending on the compound (see Table 1).

Table 1: Details of the application sent by the European Commission

Compound	Request	Matrix	
		Feed	Water for drinking
1. Manganous chloride, tetrahydrate	Re-evaluation	X	
	New use of the additive		X
2. Manganous oxide	Re-evaluation	X	
3. Manganous sulphate, monohydrate	Re-evaluation	X	
	New use of the additive		X
4. Manganese chelate of amino acids, hydrate	Re-evaluation	X	
5. Manganese chelate of glycine, hydrate	Re-evaluation	X	
	New use of the additive		X

3.1. Characterisation[13]

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

3.1.1. Manganous chloride, tetrahydrate[14]

'Manganous chloride, tetrahydrate' (Chemical Abstracts Service (CAS) No 13446-34-9) has the chemical formula $MnCl_2 \bullet 4H_2O$ (molecular weight 197.91 Da; theoretical manganese content 27.73%).

Characterisation and identity

Manganese chloride tetrahydrate is formed by reacting elemental manganese with hydrochloric acid (37%). The solution is concentrated by evaporation and the resulting manganese chloride crystals are isolated, dried and packaged.

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

[12] At the time of submission the application represented 12 companies joint as a Consortium; during the assessment, the compound (manganous carbonate) represented by company b10 was withdrawn from the dossier; therefore, it has not been considered in the current opinion.

[13] This section has been amended following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.

[14] One company involved in the application: (b6).

The product is a solid, pink in colour and odourless. Its solubility in water at 25°C is 723 g/L. It has a bulk density at 20°C of 0.800 g/cm^3.[15]

The additive contains by specification ≥ 27.0% manganese. The company provided analytical data of six batches showing a manganese content of 27–33%.[16,17]

Levels of heavy metals (Pb< 0.5 mg/kg product; Cd< 0.5 mg/kg product; Hg< 0.02 mg/kg product) and As (< 0.05 mg/kg product)[18] analysed in three batches 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 safety concern. Dioxins and the sum of dioxins and dioxin-like polychlorinated biphenyls (PCBs) were analysed in three batches showing 0.09–0.31 ng WHO-PCDD/F-TEQ/kg and 0.12–0.47 ng WHO-PCDD/F-PCB-TEQ/kg, respectively;[19] these concentrations comply with those set in Directive 2002/32/EC. The nickel content was < 15 mg/kg.[20]

Particle size distribution was characterised in four batches of the product by laser diffraction;[21,22] the percentage of particles with diameter < 50 µm ranged from 0% to 3.67%, and that of particles < 10 µm ranged from 0% to 0.02%. Dusting potential of the product (data from three batches, Stauber–Heubach method) ranged from 0.02 to 0.15 g/m^3.[23]

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 in a premixture and in a piglet feed.[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. Because of the high solubility in water of the compound, no further demonstration of homogeneity is deemed necessary.

3.1.2. Manganous oxide[25]

'Manganous oxide' (CAS No 1344-43-0) has the chemical formula MnO (molecular weight 70.94 Da; theoretical manganese content 77.39%).

Characterisation and identity

The raw materials are manganese ore and a reduction agent (coke). After selection and weighing, the raw materials are transferred into a rotate dryer for blending and elimination of the moisture in the mixture. A grinding process follows, to obtain the defined particle size, and then sieved. The sieved particles are taken to a dosage silo subsequently into an oven, where the calcination occurs at approximately 900°C. After cooling, the material goes through a final sieve; the sieved material finally goes to a storage silo. The material is then weighed and packed. The chemical composition of the product and the content of heavy metals is monitored. The plants have a Hazard Analysis Critical Control Points (HACCP) protocol implemented.

The product is a solid, brown-green coloured powder, odourless. It is insoluble in water. It has a density of 5.4 g/cm^3 and a bulk density of 1.6–1.9 g/cm^3.[26]

The additive contains by specification ≥ 60.0% manganese. The measured manganese content of the product (five batches per company) was of 60.3–62.7%. For further details regarding characterisation and identity of manganous oxide, see Appendix C, Table 3. The manganese source is a mineral ore.

[15] Technical Dossier/Section II/Annex 2-2-13.
[16] Technical Dossier/Section II/Annex 2-1-22.
[17] Technical Dossier/Supplementary Information (April 2015)/Annex_Qviii_Mn chloride 4H2O_batch to batch.pdf.
[18] Technical Dossier/Section II/Annex 2-1-40 (For heavy metals (Cd, Hg and Pb) and As).
[19] Technical Dossier/Section II/Annexes 2-1-58 (Dioxin and Sum of dioxin and Dioxin like).
[20] Technical Dossier/ Supplementary Information (April 2015)/Annex_Qii_Nickel_Mn chloride.pdf.
[21] Technical Dossier/Section II/Annex 2-2-30.
[22] Technical Dossier/ Supplementary Information (April 2015)/Annex_Qix_Mn chloride 4H2O_PSD.pdf.
[23] Technical Dossier/ Supplementary Information (April 2015)/Annex_Qix_Mn chloride 4H2O_Dusting potential.pdf.
[24] Technical Dossier/Section II/Annex 2-4-13.
[25] Two companies involved in the application: (b5) and (b7).
[26] Technical Dossier/Section II/Annexes 2-2-13 and 2-2-14.

Data on the typical composition of manganous oxide in five batches showed, further to manganous oxide (78.4–79.7%), concentrations above 1% for SiO_2 (8.5–9.2%), K_2O (2.3–2.8%), Al_2O_3 (2.1–2.3%), Fe_2O_3 (1.9–2.5%) and BaO (1.5–1.6%).[27]

Levels of heavy metals (Pb: 71–180 mg/kg product; Cd: 5.2–8.2 mg/kg product), F (90 mg/kg product) and As (34–44 mg/kg product), analysed in three to five batches of the product from each 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 safety concern. Levels for Hg reported in the first data set for company b7 (0.5–0.7 mg/kg product) appeared high (compared to other mercury thresholds, i.e. 0.1 mg/kg feed materials, 0.2 mg/kg mineral feed, 0.3 mg/kg $CaCO_3$). Upon request of EFSA, the applicant argued that the company sourced new suppliers aiming at providing a manganese oxide with an improved quality; the company provided the analysis of 22 samples, performed between 2011 and 2013, demonstrating a Hg content ≤ 0.04 mg/kg.

In addition, the nickel content was analysed in a total of five production batches (two to three batches per company), revealing a content ranging from 370 to 867 mg/kg.

Analyses of dioxins (two to four batches/company) and the sum of dioxins and dioxin-like PCBs (two to three batches/company) were carried out; concentrations (0.035–0.094 ng WHO-PCDD/F-TEQ/kg and 0.064–0.121 ng WHO-PCDD/F-PCB-TEQ/kg, respectively) comply with those set in Directive 2002/32/EC.

Particle size distribution was characterised in one batch from each company (by laser diffraction); 30.4–41.5% (v/v) of the particles had a diameter of < 52.6 µm, and 13.7–23.0% (v/v) of the particles had a diameter of < 10.8 µm. Stauber–Heubach analysis of a single batch of the product with the highest fraction of particles with diameter < 50 µm indicated a dusting potential of 4.22 g/m^3; additionally, the particle size of the dust of the product from the same company gave a result of 40.1% of particles with diameter < 50 µm.[28]

Stability and homogeneity

Stability data are not required for inorganic compounds of trace elements. Nevertheless, company b7 provided data on the shelf-life of the additive. Three batches were stored in closed bags at ambient conditions.[29] The manganese content of the product, analysed by inductively coupled plasma atomic emission spectrometry, did not change over a period of at least 3 years, indicating that manganous oxide is stable.

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 in a broiler feed.[30] 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.

3.1.3. Manganous sulphate, monohydrate[31]

'Manganous sulphate, monohydrate' (CAS No 10034-96-5) has the chemical formula $MnSO_4 \cdot H_2O$ (molecular weight 169.01g/mol; theoretical manganese content 32.48%).

Characterisation and identity

Two manufacturing processes were reported in the technical dossier. In one, manganous oxide is mixed with sulphuric acid, pressed and filtered; since precipitation occurs, the non-soluble impurities are removed. The crude manganous sulphate solution is obtained and purified by crystallisation. After dehydration and drying, the product is packed and labelled.

In the other manufacturing process, manganous oxide is first produced from manganese dioxide by calcination. Sulphuric acid is added to produce manganous sulphate. The content of iron and cadmium

[27] Technical Dossier/Supplementary Information (August 2015).
[28] Technical Dossier/Supplementary Information (April 2015)/Annex_Qiia_Mn oxide_particle_size_dust.pdf.
[29] Technical Dossier/Section II/Annex 2-4-2.
[30] Technical Dossier/Section II/Annex 2-4-14.
[31] Two companies involved in the application: (b7) and (b12).

is reduced by chemical and physical processes. The manganous sulphate solution is heated. The dried product is packed and labelled accordingly.

The whole production is performed under HACCP protocols.

The product is a solid, grey-pink crystalline powder, odourless. Its solubility in water at 20°C is 762 g/L. The density is 3.25 g/cm^3 and the bulk density ranged from 1.18 to 1.25 g/cm^3.[32]

The additive contains by specification ≥ 31.0% manganese. The measured manganese content of the product (four to six batches per company) was 31.4–34.1%. For further details regarding characterisation and identity of manganous oxide, see Appendix C, Table 4.

Levels of heavy metals (Pb < 0.5–33 mg/kg product; Cd 0.57–16 mg/kg product; Hg < 0.01-< 0.2 mg/kg product, four to six batches from three companies) and As (< 0.5–9.7 mg/kg product), measured in four to six batches from each company, and F (< 70 mg/kg product) measured in one batch from company b7, 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 safety concern. Dioxins (analysed in two to four batches per company) and the sum of dioxins plus dioxin-like PCBs (two to five batches from three companies) were 0.047–0.09 ng WHO-PCDD/F-TEQ/kg and 0.073–0.138 ng WHO-PCDD/F-PCB-TEQ/kg, respectively; these concentrations comply with those set in Directive 2002/32/EC. The nickel content was analysed in a total of seven production batches, revealing a content ranging from 23 to 891 mg/kg.

Particle size distribution was characterised in one batch from each company (by laser diffraction); up to 6.5% (v/v) of the particles had a diameter of < 52.6 µm, and up to 1.6% of the particles had a diameter of < 10.8 µm. Stauber–Heubach analysis of a single batch of the product with the highest fraction of particles with diameter < 50 µm indicated a dusting potential of 6.9 g/m^3; the particle size of the dust was requested but not provided.

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 in a broiler starter feed.[33] 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. Because of the high solubility in water of the compound, no further demonstration of homogeneity is deemed necessary.

3.1.4. Manganese chelate of amino acids, hydrate[34]

'Manganese chelate of amino acids, hydrate' is described by the applicant with the generic chemical formula $Mn(x)_{1\text{-}3}\bullet n H_2O$, where x is the anion of any amino acid derived from hydrolysed soya protein, molecular weight not exceeding 1500 Da.[35]

Characterisation and identity

The manufacturing process starts with enzymatic hydrolysis of soybean protein (under specific pH conditions). The hydrolysis process is followed by chelation with a source of manganese. The slurry is dried and packed. The applicant confirmed that only unblended products are placed on the EU market by the companies participating in this application as no anticaking/carrier or other diluents are added.[36] The applicant provided a generic process flow chart.

[32] Technical Dossier/Section II/Annexes 2-2-16 and 2-2-17.
[33] Technical Dossier/Section II/Annex 2-4-11.
[34] Seven companies involved in the application: (b1), (b2), (b3), (b4), (b8), (b9) and (b12). During the course of the evaluation, the applicant notified that companies b3 and b8 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.
[35] Commission Regulation (EC) No 1334/2003 of 25 July 2003.
[36] Technical Dossier/Supplementary Information (April 2015)/2015-04-14_TREAC_EFSA-SIn reply_Manganese_FAD-2010-0088.pdf.

The product is a solid, off-white to tan marbeled free-flowing powder, with a characteristic odour. It is reported to be dispersable in water. The product has a density of 1.4 g/cm^3 and the bulk density ranges from 0.4 to 0.7 g/cm^3.[37]

The additive contains by specification ≥ 10.0% manganese. The manganese content of 25 batches of the additive (three to seven batches from each company) was 10.2–16.7%. The applicant was requested to provide data on the proportions of manganese chelate and any inorganic manganese. The applicant developed an indirect method (based on Fourier Transform Infrared (FTIR) spectroscopy) to estimate the binding of manganese to amino acids. The values were quantified by comparison with a calibration curve. The results of this *in-house* method, although validated, are not considered fully reliable but allow the conclusion that between 35% and 100% of total manganese occurs as chelates.

Analytical data of nine batches (company/ies not specified) was provided showing the percentage of molecules with molecular weight ≤ 1500 Da. Considering all values, up to 30% of the molecules exceeded 1500 Da. The FEEDAP Panel notes that the specification of the applicant on the molecular 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 manganese 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 four batches (one batch from each of four companies) of the additive revealed a content of 2.9–8.9% moisture, and on an *as is* basis: 24.1–31.7% crude protein (nitrogen × 6.25), 0.0–1.4% lipids, 1.4–2.6% crude fibre and 26.4–46.4% ash; the mineral fraction consists of 0.3–0.7% calcium, 6.8–9.7% sulphur, 0.1–2.6% sodium, 1.1–1.3% potassium and 0.2–0.4% phosphorus.[38,39,40,41] For further details regarding characterisation and identity of manganese chelate of amino acids, hydrate, see Appendix C, Tables 5 and 6.

Levels of heavy metals (Pb < 0.05–20 mg/kg and Cd < 0.1–7 mg/kg, at least one batch of the product from each company; Hg < 0.005– < 0.5 mg/kg, 10 batches from four companies), As (< 0.1–1 mg/kg at least one batch from each company) and fluorine (F) (< 0.10 mg/kg, three batches from one 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 safety concern. Dioxins (analysed in a total of 10 batches from the five companies) and the sum of dioxins plus dioxin-like PCBs (analysed in one to three batches from four companies) were 0.015–0.16 ng WHO-PCDD/F-TEQ/kg and 0.016–0.318 ng WHO-PCDD/F-PCB-TEQ/kg, respectively; these concentrations comply with those set in Directive 2002/32/EC. The nickel content was analysed in a total of seven production batches (at least one batch per company) revealing a content ranging from 11 to 472 mg/kg.

The analysis of mycotoxins in seven batches of the product from five companies did not raise concern (aflatoxin B1 and ochratoxin A were found at a maximum concentration of 0.35 µg/kg and below 0.5 µg/kg, respectively). In the batches stored from 1 to 40 months, the content of moulds, yeasts and total coliforms was below 100 CFU (colony-forming units)/g; Salmonella was absent in 25 g of the product.

Particle size distribution was characterised in one to four batches per company by laser diffraction or sieving. The fraction of particles with diameter below 50 µm ranged from approximately 4% to 73%, and that below 10 µm ranged from 0% to 22.5%. Dusting potential (Stauber–Heubach method) was determined only in five batches of the product identified as having the highest percentage of particles < 50 µm, and was up to 0.88 g/m³; the manganese content of the dust was analysed in four batches

[37] Technical Dossier/Section II/Annex 2-2-1 (b1), Annex 2-2-2 (b2), Annex 2-2-4 (b4), Annex 2-2-6 (b9) and Annex 2-2-7 (b12).
[38] Technical Dossier/Supplementary Information (April 2015)/Annex_Qxx_Mn chelate CoA_Company b1.pdf
[39] Technical Dossier/Supplementary Information (April 2015)/Annex_Qxx_Mn chelate CoA_Company b2.pdf
[40] Technical Dossier/Supplementary Information (April 2015)/Annex_Qxx_Mn chelate CoA_Company b4.pdf
[41] Technical Dossier/Supplementary Information (April 2015)/ Annex_Qxx_TREAC_Company b12_MANGANESE_Composition.pdf

and showed an average of 10.50% (range 9.8–11.3%).[42] Due to the small amount of dust collected, the particle size distribution of the dust could not be determined.[43]

Stability and homogeneity

No stability (including shelf-life) data were provided for the manganese chelate of amino acids, hydrate, in particular concerning the maintenance of the specific bonds of manganese 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 was shown to be stable to microbial contamination for 1–40 months (see Characterisation and identity).

Experimental data on the capacity for homogeneous distribution of the additive were provided by company b1. Manganese content was determined in 15 samples of a complementary feed for horses containing manganese chelate of amino acids, hydrate; the mean concentration was 411 mg total Mn/kg, the coefficient of variation (CV) was 0.97%.[44]

3.1.5. Manganese chelate of glycine, hydrate[45]

'Manganese chelate of glycine, hydrate' is derived from synthetic glycine mixed with a manganese salt. It has the generic formula $Mn(x)_{1-3} \bullet nH_2O$, where x is the anion of glycine.

Characterisation and identity

To produce manganese chelate of glycine, hydrate, a manganese salt (sulphate, carbonate) or any other source of manganese is mixed with synthetic glycine; the raw materials could be of any source that complies with EU Regulations. 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.

The product is a solid, odourless, beige to pink free-flowing powder. Its solubility in water is >10 g/L. The product has a density of 0.97 g/cm^3 and the bulk density ranges from 0.79 to 0.92 kg/m^3.[46]

The additive contains by specification ≥ 15.0% manganese. The manganese content of 34 batches of the additive, at least three batches from each company, ranged from 15.8% to 27.0% – the large range results primarily from the different ratios of manganese to glycine. For further details regarding characterisation and identity of manganese chelate of glycine, hydrate, see Appendix C, Table 7.

Additional data on 10 batches showed that the manganese content ranged from 16.2% to 22.7%, extractable glycine from 32.2% to 51.0%, sulphur from 9.5% to 15.2% and moisture from 0.4% to 5.2%. The molar ratio of glycine to manganese in the different products ranged from 3.1 to 1.4[47] (see also Appendix C, Table 8).

Levels of heavy metals (Pb < 0.5–34 mg/kg, Cd < 0.5–4 mg/kg, analysed in at least three batches per company; Hg < 0.5 mg/kg, three batches each from three companies) and As (<001–1 mg/kg, one to five batches per 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 safety concern. Dioxins (analysed in one to five batches from each company) and the sum of dioxins plus dioxin-like PCBs (one to five batches from three companies) were 0.018–0.17 ng WHO PCDD/F-TEQ/kg and 0.0298–0.153 ng WHO-PCDD/F-PCB-TEQ/kg, respectively; these concentrations comply with those set in Directive 2002/32/EC. Nickel was analysed in one or two batches per company, showing a range of 53–501 mg/kg.

[42] Technical Dossier/Supplementary Information (April 2015)/Annex_Qxvi_Mn AA chelate_Mn in dust.pdf.
[43] Technical Dossier/Supplementary Information (April 2015)/Annex_Qxvi_Mn AA chelate_PSD of dust.pdf.
[44] Technical Dossier/Section II/Annex 2-4-8.
[45] Four companies involved in this application: (b2), (b4), (b9) and (b11).
[46] Technical Dossier/Section II/Annex 2.2.8 (b2), Annex 2.2.9 (b4), Annex 2.2.10 (b9), Annex 2.2.11 (b11).
[47] Technical Dossier/Supplementary Information (April 2015).

Microbial contamination was analysed in one batch at 0, 24 and 48 h after dilution in water.[48] At all time points studied, total aerobic count, moulds and yeasts were below 1 CFU/g, and Salmonella was not detected in 25 g sample of the product.

Particle size distribution was characterised in one to three batches per company by laser diffraction. The products differed markedly in their particle size distribution. For one group of additives 100% of particles showed a diameter < 50 µm, with up to 93.5% of particles < 10 µm. The fraction < 50 µm in the other group of additives amounted up to approximately 11%; the fraction < 10 µm ranged from approximately 0.8 to 4%. 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 2.23 g/m³. The particle size of the dust was analysed in one batch of the additive of company b2 showing 53.4% of particles of diameter < 10 µm. The manganese content of the dust was not provided, although requested.

Stability and homogeneity

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

Experimental data on the capacity for homogeneous distribution of the additive were provided by company b11. Manganese content was determined in eight samples of a premixture containing manganese chelate of glycine, hydrate; the mean concentration was 35 mg total Mn/kg, indicating a CV of 5.7%.[49]

3.1.6. Physico-chemical incompatibilities

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

The applicant provided data on the influence of manganese chelate of glycine, hydrate, or manganese sulphate monohydrate (10 mg Mn/L) on the stability of vitamins A, D_3 and E in a liquid vitamin–mineral mixture, also containing iron, copper and zinc. The vitamin concentration was compared to a control mixture, without addition of trace elements, after 2 weeks of storage. The three vitamins were stable in the presence of the trace element sources tested.[50]

3.1.7. Conditions of use

All manganese compounds under application are intended to be administered to feedingstuffs via a premixture. The use of three compounds (manganous chloride, tetrahydrate; manganous sulphate, monohydrate; manganese chelate of glycine, hydrate) is also applied for use in water for drinking for all animal species and categories, except fish (for details, see Table 2).

The maximum manganese contents proposed for water for drinking are derived from the maximum contents set for complete feed by a factor (ratio of water/feed intake) of 7 for milk replacer, of 10 for ovine and of 2–3 for other species (see Table 2). It is noted that the provided manganese calculations for use in water for drinking do not consider the background manganese content in feed.

[48] Technical Dossier/Supplementary Information (April 2015)/Annex_Qxxiv_Mn glycinate_stability water_microbio (T0).pdf. Annex_Qxxiv_Mn glycinate_stability water_microbio (T24).pdf. Annex_Qxxiv_Mn glycinate_stability water_microbio (T48).pdf.
[49] Technical Dossier/Section II/Annex 2.4.9.
[50] Technical Dossier/Section II/Annex 2.4.15.

Table 2: Maximum contents of manganese in complete feed and water for drinking, as proposed by the applicant

Animal species/category	Maximum total manganese content	
	Complete feed (mg/kg)	Water for drinking(mg/L)[(a)]
Fish	100	
Pigs	150	68
Ovine		15
Equine		75
Poultry		75
Bovine: Milk replacer		21
Other bovine		50

(a): The proposed manganese content in water assumes that no manganese is administered via feed (including feed background).

3.2. Safety

3.2.1. Safety for the target species

Tolerance studies are not required for compounds of trace elements already authorised (Regulation (EC) No 429/2008). The assessment of manganese safety for the target species is based on a previous opinion of the SCAN (EC, 2002) and on FEEDAP Panel opinions (EFSA, 2009a, 2010a, EFSA FEEDAP Panel 2013a, 2013b, 2013c).

Manganese is an essential trace element in livestock nutrition. Its essentiality in livestock nutrition (poultry) became evident in the 1930s (Lyons and Insko, 1937; Wilgus et al. 1937; Schaible et al., 1938).

Manganese has a long history of safe use in animal feeding. In general, manganese is considered to be one of the least toxic essential trace elements for farm animals. Depressed iron status and haematological changes are the most common signs of manganese toxicosis, also observed in animals fed adequate levels of iron (NRC, 2005). The National Research Council defined in 2005 maximum tolerable levels (MTL, in mg/kg DM) for ruminants and poultry (2000), pigs (1000) and horses (400), whereas no MTL could be derived for fish because of insufficient data (NRC, 2005). The current authorised maximum total contents in complete feed in the EU (100 mg/kg for fish and 150 mg/kg for other animal species) provide a sufficient margin of safety to the MTLs as well as a sufficient margin of safety to the allowances, with estimated requirements for chicken and turkeys for fattening of 60 mg/kg, for laying hens of 30 mg/kg, for pigs and cattle of 15–25 mg/kg (GfE, 1999, 2001, 2008; NRC, 1994, 2001; Suttle, 2010).

Regarding the use of some of the compounds in water for drinking, it appears that the maximum manganese concentration in water for drinking as proposed by the applicant (Table 2) was derived from calculations in which no manganese background of feed and a low water intake of animals (mainly only 2 L/kg feed) were considered. For poultry, pigs and calves (fed milk replacer), 50, 33 and 18 mg Mn/L water for drinking were derived, respectively, as safe maximum concentrations; the corresponding values for dairy cows are 2,405 mg Mn/day and for cattle for fattening 240 mg Mn/100 kg bw (body weight) (for further details, see Appendix D).

The FEEDAP Panel notes that the above calculated maximum manganese concentrations and daily doses that could be applied via water for drinking can only be used if feed not supplemented with manganese is given. It is also noted that the calculations do not consider the manganese content of water for drinking. Consequently, the FEEDAP Panel reiterates its recommendation that compounds of trace elements should generally not to be used via water for drinking (EFSA, 2010b).

As the additives contain nickel as a contaminant, the FEEDAP Panel assessed the impact of nickel on safety for the target species. According to the NRC (2005), horses, fish and rodents are the most sensitive animal species to nickel with a MTL of 50 mg/kg feed. In the worst-case scenario, the nickel that would be added to the feed if supplemented with manganese chelate of amino acids, hydrate would amount to less than 0.5 mg/kg feed for horses. Together with the background nickel in feed (i.e. 4 or 9 mg/kg DM feed; Nicholson et al., 1999; Van Paemel et al., 2010; EFSA CONTAM Panel,

2015), the total nickel would then amount up to 9.44 mg Ni/kg feed, corresponding to approximately 20% of MTL of the sensitive animal species. Therefore, the nickel content of the additives does not represent a safety concern for the target species.

Interactions

Manganese and iron compete for absorption sites. Fibre, phytate, calcium, phosphorus and magnesium may also interfere with manganese absorption. The manganese compounds under assessment are not expected to show any relevant interactions with feed components other than those well recognised for manganese.

Conclusions on the safety for the target species

The FEEDAP Panel concludes that all the manganese compounds under application are considered safe for all animal species/categories, provided that the current maximum total contents of manganese authorised in feed (100 mg/kg complete feedingstuffs for fish and 150 mg/kg for other species) are respected.

3.2.2. Safety for the consumer

Metabolic and residue studies

Manganese is absorbed through the small intestine by non-saturable simple diffusion (Bell et al., 1989) or by a carrier-mediated transport mechanism with high affinity and low capacity (Garcia-Aranda et al., 1983). Typical apparent absorption may be in the range of 3–5%; however, values as high as 40% have been observed in veal calves with a supply of only 0.6 mg Mn/kg milk replacer (Kirchgessner and Neese, 1976). Absorption in poultry is lower, explaining the higher requirement. Intestinal absorption of manganese is also inversely related to iron intake (Lönnerdal, 1997). Manganese absorbed into portal circulation is transported by α_2-macroglobulins or albumin to the liver. The major portion of manganese in the liver is secreted into bile. Consequently, manganese is primarily excreted via faeces. Urinary excretion of manganese is only a minor route of excretion; it reflects endogenous homeostatic regulation and is not related to the dietary intake (NRC, 2005).

The highest concentrations of manganese are found in the liver, pancreas and kidney and the lowest levels occur in bone and fat. Nevertheless, manganese deposition in any of these tissues is not considered a valuable indicator of manganese availability in the gut (Jongbloed et al., 2002).

Manganese deposition studies

Residue/deposition studies are not required for an already approved compound of trace elements.

The applicant provided a report of publications comparing organic and inorganic manganese compounds based on a total of 14 experiments performed with manganese combined with other minerals (seven with Cu/Fe/Zn, six with Cu/Zn and one Cu/Fe). The table in Appendix E summarises the data of these studies on manganese deposition in liver, muscle or eggs.

In four experiments (Mabe et al, 2003; Ao and Pierce, 2006; Huyghebaert, 2006; Dobrzanski et al., 2008), organic manganese sources did not influence the manganese content of the egg (total, egg yolk and egg albumin) differently to inorganic sources.

Only one study (Kinal et al., 2007) comparing the effect of organic and inorganic manganese sources on manganese content of milk was submitted; no differences were found.

Another comparison of inorganic and organic manganese sources was made regarding manganese in liver (one study in broilers (Bao et al., 2007), two in piglets (Schiavon et al., 2000; Martin et al., 2011) and two in dairy cows (Olson et al., 1999; Nocek et al., 2006)). The results showed no evidence for different deposition of manganese in liver between the two manganese sources in four of the five studies submitted. Nevertheless, one study (Martin et al., 2011) showed that manganese in liver was increased when a diet with an organic manganese source was fed.

Four studies reported manganese deposition in muscle following supplementation with organic and inorganic sources. One study in piglets (Martin et al., 2011) showed that deposition in muscle

occurred at very low level upon supplementation with either an organic or an inorganic source, albeit manganese deposited in muscle was higher with the organic source. One study in chickens for fattening (Petrovic et al., 2010) reported no differences in manganese muscle deposition between organic and inorganic sources. Two studies in rainbow trout were available, yielding different results: Apines et al. (2003) observed a twofold increase in whole body manganese when supplementation was done by the organic source; however, this finding was not confirmed by a subsequent publication of the same group (Apines et al., 2004). The overall evidence suggests that muscle tissue is not an important deposition site for manganese, irrespective of the supplementation source.

Toxicological profile of manganese. Toxicological reviews

Manganese is essential in the nutrition of both animals and humans. The FEEDAP Panel reviewed the relevant literature and considered several previous toxicological assessments of manganese. It is noted that there are relatively limited data available on oral toxicity in laboratory animals and humans.

As is the case for all transition metals, long-term exposure to high doses of manganese poses a risk. The toxicology of manganese has been reviewed by the Scientific Committee on Food (SCF) (EC, 2000), the UK Expert Group on Vitamins and Minerals (EVM 2002, 2003), the US Environmental Protection Agency (EPA, 2003), the UK Institute of Environment and Health (IEH, 2007) and most recently the US Agency for Toxic Substances and Disease Registry (ATSDR, 2012). The toxicology of manganese has been recently reviewed by Lucchini et al. (2015). There is a consensus that the main toxic effects of manganese in humans concern the nervous system, the respiratory system (inhalatory exposure) and, to a lesser extent, the reproductive system.

Chronic severe toxicity is primarily associated with effects on the central nervous system (CNS), especially through inhalation and long-term exposure, and is considered much more relevant than acute effects (Huang et al., 1989; cited by IEH, 2007). Common neurological symptoms arising from chronic manganism tend to occur in phases and start with anorexia, weakness and apathy, followed by a second phase of hallucinations, delusions and insomnia. During the later stages of chronic toxicity, Parkinson-like symptoms such as tremor and muscle rigidity take place. Although manganism and true idiopathic Parkinson disease cause very similar deficits within the CNS, they differ in the neurotransmitters upon which they act; manganese toxicity results principally in the degeneration of γ-aminobutyric acid-ergic neurons in the globus pallidus whilst Parkinson disease is more associated with the dopaminergic neurons in the basal ganglia (Roth, 2006; cited by IEH, 2007).

Manganese aggregates into non-haem iron regions of the brain such as the globus pallidus, substantia nigra and subthalamic nuclei (Aschner et al., 2007). Although the precise mechanisms by which manganese induces toxic effects within the CNS are a matter of continuing debate, there are a number of reports which highlight possible interactions between manganese and other trace elements such as iron, copper and aluminium (IEH, 2007). Postulated mechanisms of manganese-induced neurotoxicity include (i) increased production of reactive oxygen species (Cohen, 1984; cited by IEH, 2007); (ii) neuronal degeneration by means of activation of glutamate-gated channels (Brouillet et al., 1993; cited by IEH, 2007); (iii) manganese in a divalent (or higher) oxidation state exerting toxicity on dopamine (Archibald and Tyree, 1987; cited by IEH, 2007); (iv) dopamine oxidation by manganese causing oxidative DNA damage (Oikawa et al., 2006, cited by IEH, 2007); and (v) production of 6-hydroxydopamine (or other toxic catecholamines; Graham, 1984; cited by IEH, 2007). Another publication focuses on dopamine oxidation and mitochondrial damage as the main mode of action (Farina et al., 2013).

There are association studies suggesting that, as in animal models, excess manganese exposure in humans can lead to reproductive toxicity, resulting in decreased fertility and increased foetal abnormalities (Lauwerys et al., 1985; cited by IEH, 2007; Crossgrove and Zheng, 2004; cited by IEH, 2007). For example, in one study, manganese-exposed male workers were found to have fewer children than others (Lauwerys et al., 1985; cited by IEH, 2007). However, other studies did not find the same effect (IEH, 2007).

The results of *in vitro* studies show that at least some chemical forms of manganese have mutagenic potential (ATSDR, 2012). The issue of manganese genotoxicity has been recently reconsidered by Lima et al. (2011); available data confirm that manganese can exert genotoxic effects in human lymphocytes *ex vivo* without any marked concurrent cytotoxicity. Likely mechanisms include oxidative

damage (as a transition metal, manganese is a potential reactive oxygen species inducer) and the interaction with DNA polymerases and other proteins involved with DNA-dependent processes (Wafik et al., 1984). The results of *in vivo* studies in rodents are inconsistent, and in its assessment of manganese, ATSDR (2012) was unable to draw an overall conclusion about the possible genotoxic hazard to humans from exposure to manganese compounds.

Information on *in vivo* chronic toxicity of manganese comes from the National Toxicology Program (NTP), which conducted two chronic studies in rats and mice (NTP, 1993). F344/N rats were exposed orally for 2 years to manganese from manganous sulphate at 60, 200 or 465 mg/kg bw (males) or 70, 230 or 714 mg/kg bw (females). Under the conditions of this 2-year feeding study, there was no evidence of carcinogenic activity of manganese (II) sulphate monohydrate in male or female rats (NTP, 1993). In a 2-year study in B6C3F1 mice, the animals were exposed orally to 160, 540 or 1,800 mg Mn/kg bw (males) or 200, 700 or 2,250 mg Mn/kg bw (females). There was equivocal evidence[51] of carcinogenic activity of manganese (II) sulphate monohydrate in male and female mice, based on a marginally increased incidence of thyroid gland follicular cell adenoma and a significantly increased incidence of follicular cell hyperplasia. The ingestion of diets containing manganese (II) sulphate monohydrate was associated with focal squamous hyperplasia of the forestomach in male and female mice, and ulcers and inflammation of the forestomach in male mice. EVM (2002) echoed the conclusions by NTP (1993) and stated that 'Chronic carcinogenicity studies of manganese sulphate in mice and rats were essentially negative, with equivocal evidence of carcinogenicity being observed in mice only'. There is no epidemiological or other evidence suggesting that manganese causes cancer in humans (ATSDR, 2012; Lucchini et al., 2015).

Assessment of consumer safety

See previous FEEDAP Panel opinions on re-evaluation of manganese compounds as feed additives (EFSA FEEDAP Panel, 2013a, 2013b, 2013c).

Conclusions on safety for consumers

The usual intake levels of dietary manganese do not appear to be associated with any adverse health effects in humans, albeit it is advisable that oral exposure to manganese should not increase over the background intake. Tissues and products of animal origin generally provide a low contribution to the overall manganese dietary intake and are not influenced to any important degree by the supplementation of feeds at the current maximum authorised levels in the EU, irrespective of whether the source is inorganic or organic. Moreover, due to the long history of use of manganese supplementation of feed for food-producing animals, the dietary intake data in humans are expected to include foods derived from animals reared with manganese-supplemented feed. It is concluded that the use of the manganese compounds under assessment in animal nutrition is of no concern for the safety of consumers, provided that the current maximum total contents of manganese authorised in feed are respected.[52]

3.2.3. Safety for users/workers

No specific studies were provided by the applicant regarding the toxicity of the additives for users/workers.

Effects on skin and eyes

Two of the compounds under assessment, manganous sulphate and manganous chloride, react acidic at contact with water; these salts are considered as irritants after contact with eyes and mucosae, but not irritating or sensitisers to skin (Ikarashi et al., 1992; Basketter et al., 1999; ATSDR, 2012; Lucchini et al., 2015).[53] Another additive, the insoluble manganous oxide, is not a skin irritant or skin sensitiser, but may mechanically irritate the eyes after exposure (ATSDR, 2012).[54] The limited

[51] 'Equivocal evidence' of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase in neoplasms that may be chemical related (NTP, 1993).
[52] 100 mg total Mn/kg for fish and 150 mg total Mn/kg for other species.
[53] Technical Dossier/Section II/MSDS Manganous chloride, tetrahydrate; MSDS Manganous sulphate, monohydrate.
[54] Technical Dossier/Section II/MSDS Manganous oxide.

database available[55] indicates that the manganese chelate of glycine, hydrate and the manganous chelate of amino acid, hydrate may irritate skin and eyes and, for the latter, owing to its proteinaceous nature, should be considered as a skin sensitiser. The presence of nickel in the additives may induce contact dermatitis in workers.

Effects on the respiratory system

Manganese is a recognised workplace toxicant upon inhalation exposure. Inhalation of manganese can result in pulmonary oedema and tracheobronchitis (Nemery, 1990; cited by IEH, 2007). Furthermore, inhaled manganese is particularly hazardous as it can be transported directly to the brain, bypassing the intestinal absorption control and liver metabolism (ATSDR, 2012). Some studies suggest that manganese inhalation can result in adverse cognitive effects, such as attention deficit and amnesia (ATSDR, 2012) as well as parkinsonism-like symptoms (Park, 2013). Individuals subject to prolonged occupational exposure to high levels of manganese are considered to be most at risk. Thus, welders, miners and other metal industry workers have increased incidence of pulmonary conditions such as pneumonia and bronchitis (Saric and Piasek, 2000; cited by IEH, 2007). The lung epithelium functions as a barrier to infection and its ability to respond to foreign antigens is disrupted in the presence of manganese along with many other transition metals. In a study by Roth and Garrick (2003; cited by IEH, 2007), manganese was regarded as the second most important metal, after copper, in causing inflammation of the lung tissue. This inflammation is thought to occur by means of cytokine release (interleukins) as opposed to being immunoglobulin E mediated.

All manganese compounds under assessment have fractions of particle sizes < 50 µm (i.e. inhalable) exceeding 1%. The dusting potentials are in the range between 0.15 g/m^3 (manganese chelate of amino acids) and 6.9 g/m^3 (manganous sulphate monohydrate). Therefore, exposure of workers/users by inhalation when handling the product is likely to be high. Moreover, some of the additives are water soluble and can potentially be absorbed by the epithelium of the upper respiratory tract to become available to the circulatory system. The human body has an effective control system which normally prevents the intestinal absorption of an excess of manganese from the diet; however, this system is bypassed upon inhalation exposure of soluble manganese compounds.

The Health and Safety Executive (HSE) of the UK and the Occupational Safety and Health Administration (OSHA) of the US have set an occupational exposure standard for dust from manganese and its compounds (HSE, 2003; OSHA, 2007) of 5 mg Mn/m^3. In the 2013 edition of its TLVs and Biological Exposure Indices publication, the American Conference of Governmental Industrial Hygienists reduced the threshold limit value (TLV) for inhalable manganese particles to 0.1 mg/m^3. The corresponding value for respirable fractions of manganese was set to 0.02 mg/m^3. The new TLVs do not distinguish between the form of manganese found in welding fume and other forms of manganese and are thus relevant for the additives under consideration. Owing to the manganese content in the additives, the manganese concentration in the dust produced by the additives in the Stauber–Heubach apparatus can greatly exceed the TLV value (manganese chloride: 40 mg/m^3; manganous oxide: 2,530 mg/m^3; manganese sulphate, monohydrate: 2,140 mg/m^3; manganese chelate of amino acids, hydrate: 90 mg/m^3; manganous chelate of glycine, hydrate: 330 mg/m^3). Therefore, exposure of persons handling the additives poses a risk of inhalation toxicity.

The FEEDAP Panel considered whether the presence of nickel in the additives under evaluation would pose additional concerns to the safety of users. The nickel content of the additives under assessment was provided and reached concentrations close to 900 mg/kg of the additive for manganous sulphate, monohydrate and manganous oxide. Inhalation of soluble nickel can cause pulmonary toxicity, resulting in bronchitis, fibrosis and lung cancer in humans. The proposed occupational exposure limit (OEL) for the inhalable fraction of water-soluble nickel is 0.01 mg/m^3 (EC, 2011). Considering the dusting potential and the nickel content of the additives, the nickel released in the dust from handling manganous sulphate, monohydrate and manganese chelate of glycine hydrate is more than two orders of magnitude higher than the proposed OEL, that of chelate of amino acids, hydrate one order of magnitude.

Conclusions on safety for the users/workers

[55] Technical Dossier/Section II/MSDS Manganese amino acid chelate; MSDS Manganese glycinate chelate.

Manganese chloride, tetrahydrate, manganous sulphate, hydrate and manganous oxide are not irritants to skin or dermal sensitisers; these three compounds should be considered as eye irritants. It is prudent to regard the manganese chelate of glycine, hydrate, and the manganous chelate of amino acid, hydrate, as irritants to skin and eyes, and the amino acid chelate as a dermal sensitiser.

Due to the high dusting potential, all manganese compounds under assessment can cause respiratory toxicity in persons handling the additive.

The nickel content in the dust of manganous sulphate, monohydrate, manganese chelate of glycine hydrate and the manganese chelate of amino acids, hydrate represents a risk by inhalation. Moreover, the presence of nickel in all additives may induce contact dermatitis in users.

3.2.4. Safety for the environment

Manganese is the second most abundant (after iron) transition element in the Earth's crust, with an estimated global average in soil of 437 mg/kg (Forum of European Geological Surveys, FOREGS),[56] ranging from 40 to 900 mg/kg (Lucchini et al., 2015). The median concentration of manganous oxide in 845 samples of topsoil collected throughout Europe for the FOREGS survey was 650 mg/kg and the manganese content of the acid-soluble fraction (*Aqua regia*) contained 382 mg/kg, with a range of < 10–6,480 mg/kg (FOREGS). The behaviour of manganese in soil is very complex and is controlled by different environmental factors, of which pH-Eh conditions are the most important (Kabata-Pendias 2001). Mn^{2+} appears to have low affinity for organic ligands (Lazerte and Burling, 1990; Chiswell and Zaw 1991). However, whilst the Mn^{2+} (aqueous) ion is readily soluble, manganese in soil is not very mobile, because Mn^{3+} and Mn^{4+} form insoluble hydrous oxides, especially under oxidising conditions.

Concentrations of dissolved manganese in European stream waters from the FOREGS survey range from < 0.05 to 698 µg/L, with a median value of 15.9 µg/L (*n*= 804). The lowest values of dissolved manganese (< 1.7 µg/L) are found in central and northern Sweden, in central and south Norway, and in western Scotland and western England on Caledonian terrains (FOREGS).

Environmental safety from use in feeds for terrestrial farm animals

Based on the calculation method provided in the technical guidance for assessing the safety of feed additives for the environment (EFSA, 2008b), the highest theoretical addition of manganese in soil (PEC_{soil}) from animal feeds is around 3 mg/kg (lambs) after a 1-year application of manure assuming that 100% of a dose will be excreted. As the median content of manganese in European soil is over two orders of magnitude higher than this value, the use of manganese in animal feeds at the legislated inclusion levels is not expected to pose a risk to the soil compartment.

Environmental safety from use in aquaculture feeds

Using its technical guidance for assessing the safety of feed additives for the environment (EFSA, 2008b), the FEEDAP Panel calculated the worst-case concentrations in the environment resulting from the supplementation of fish feeds with the manganese compounds under assessment at the total level of 100 mg Mn/kg. When fed to fish in sea cages, the sediment under the cage is considered the compartment of concern (EFSA, 2008b). The PEC_{sed} was calculated to be 21.2 mg/kg wet weight, which substantially exceeds the threshold for Phase I assessment (10 µg/kg). However, manganese concentrations in top sediment in marine and estuarine environments vary widely from 10 to at least 4,000 mg/kg, which in all cases by far exceeds that maximally emitted from aquaculture (Sadiq and Zaidi, 1985; Cahill and Unger, 1993; Galasso et al., 2000; Sahli et al., 2011). For land-based aquaculture operations, such as ponds and raceways, the surface water downstream of the fish farm is considered the most sensitive compartment (EFSA, 2008b). The PEC_{swaq} was calculated to be 0.07-0.25 µg /L, depending on the species. This concentration slightly exceeds the trigger value, but is yet two orders of magnitude lower than the median concentration of manganese in European freshwaters (FOREGS).

Conclusions on environmental safety

[56] Available online: http://weppi.gtk.fi/publ/foregsatlas/

The use of the manganese compounds under assessment (manganous chloride, tetrahydrate; manganous sulphate, monohydrate; manganous oxide; manganese chelate of amino acids, hydrate; manganese chelate of glycine, hydrate) in animal nutrition for all animal species is safe for the environment, provided that the current maximum total contents of manganese authorised in feed are respected.

3.3. Efficacy

No efficacy studies are required for compounds of trace elements already authorised as feed additives.

The use of the manganese compounds under assessment in animal nutrition is extensively documented in the scientific literature and summarised by McDowell (2003) and Suttle (2010). They are recognised as efficacious sources of manganese in meeting animals' requirements.

3.4. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[57] and good manufacturing practice.

4. Conclusions

The manganese compounds under consideration are: manganous chloride, tetrahydrate; manganous sulphate, monohydrate; manganous oxide; manganese chelate of amino acids, hydrate and manganese chelate of glycine, hydrate. The safety assessment is based on the assumption that the current maximum total contents of manganese authorised in feed are respected.

All manganese compounds under application are considered safe for all animal species/categories.

The mean manganese intake of the European population includes already the manganese from animal products and does not pose a toxicological concern. The effect of dietary manganese fed to animals on tissue concentrations is limited. Supplementation of feed with the manganese compounds under assessment would consequently not affect consumer exposure and is of no concern for consumer safety.

All manganese compounds are considered as eye irritants, the manganese chelate of glycine and the manganese chelate of amino acids as irritants to skin and the latter one as dermal sensitiser. However, the presence of nickel in all additives may induce contact dermatitis. Exposure to manganese in dust of all additives and to nickel (except manganous chloride and manganous oxide) poses a risk to users by inhalation.

The use of the manganese compounds under assessment in animal nutrition for all animal species is not expected to pose a risk to the environment.

The manganese compounds under assessment are recognised as efficacious sources of manganese in meeting animals' requirements.

5. Recommendations

For all manganese compounds, monitoring of the nickel content, and for manganous oxide monitoring of mercury content, should be included in the HACCP plan. Attempts should be made for a continuous reduction in the nickel content.

Concerning the manganese chelate of amino acids, hydrate, the FEEDAP Panel identified in a previous assessment (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 dalton'. Data from different companies reveal that up to 30% of the molecules may have a molecular weight exceeding 1500 Da. A previous assessment of manganous chelate of amino acids, hydrate considered

[57] 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.

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 dalton'. The Panel recommends to keep this definition further on 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 dalton'; 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 manganese chelates into two groups, for which the following names and characteristics are proposed:

- Manganese chelate of amino acids, hydrate: Not more than 10% of the molecules exceeding 1500 Da
- Manganese chelate of protein hydrolysates: Between 10% and 50% of the molecules exceeding 1500 Da

  In both the above cases, at least 50% of the manganese should be present as chelate.

For both groups, the general formula of $Mn(x)_{1\text{-}3} \bullet n H_2O$ (x is the anion of any amino acid from soya protein hydrolysate) is proposed.

For user safety, the workplace concentration of manganese should not exceed 0.02 mg Mn/m^3.

Maximum contents for total manganese in feed are set by legislation. The conclusions of the FEEDAP Panel on the safety of the five manganese compounds assessed for the target animals, the consumer and the environment are only valid if these maximum contents are strictly considered in feed formulation. As manganese is routinely supplemented to feed, only a small amount, if any, could be administered additionally via water for drinking. Exact dosing in water for drinking can only be made if the total dietary manganese content is known, which is normally not the case. The FEEDAP Panel therefore does not recommend the use of manganese compounds via water for drinking. The simultaneous use of feed and water both supplemented with manganese should be avoided. Considering the mentioned caveat, the following concentrations/daily doses comply with the maximum content authorised for feed: poultry, 50 mg Mn/L; pigs, 33 mg Mn/L; calves (fed milk replacer), 18 mg Mn/L; dairy cows, 2405 mg Mn/day; cattle for fattening, 240 mg Mn/100 kg bw per day.

The FEEDAP Panel stresses the general need for analytical methods to quantify the organic compounds of trace elements in feed.

Documentation provided to EFSA

1. Dossier Manganese (E5). Nutritional feed additive. Compounds of trace elements. February 2010. Submitted by TREAC EEIG (Trace Elements Authorisation Consortium).

2. Dossier Manganese (E5). Nutritional feed additive. Compounds of trace elements. Supplementary information. April 2015. Submitted by FEFANA asbl.

3. Dossier Manganese (E5). Nutritional feed additive. Compounds of trace elements. Supplementary information. August 2015. Submitted by FEFANA asbl.

4. Evaluation report of the European Union Reference Laboratory for Feed Additives on the methods(s) of analysis for Manganese (E5).

5. Comments from Member States received through ScienceNet.

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Abbreviations

ATSDR	Agency for Toxic Substances and Disease Registry
As	Arsenic
bw	body weight
CAS	Chemical Abstracts Service
Cd	Cadmium
CFU	colony-forming units
CNS	central nervous system
Cu	Copper
CV	coefficient of variation
DM	dry matter
EC	European Commission
EEC	European Economic Community
EURL	European Union Reference Laboratory
EVM	Expert Group on Vitamins and Minerals
F	fluorine
Fe	iron
FEEDAP	Panel on Additives and Products or Substances used in Animal Feed
FEFANA	EU Association of Specialty Feed Ingredients and their Mixtures
FOREGS	Forum of European Geological Surveys
HACCP	Hazard Analysis Critical Control Points
Hg	mercury
HSE	Health and Safety Executive
IEH	Institute of Environment and Health
MTL	maximum tolerable level
Mn	manganese
Ni	nickel
NTP	National Toxicology Program
NRC	National Research Council
OEL	occupational exposure limit
OSHA	Occupational Safety and Health Administration
Pb	lead
PCBs	polychlorinated biphenyls
PCDDs	polychlorinated dibenzo-para-dioxins
PCDFs	polychlorinated dibenzofurans
PEC_{soil}	predicted environmental concentrations in soil
PEC_{sw}	predicted environmental concentrations in surface water

SCAN	Scientific Committee on Animal Nutrition
SCF	Scientific Committee on Food
TEQ	toxic equivalent factor
TLV	threshold limit value
TMR	total mixed ration
TREAC EEIG	Trace Elements Authorisation Consortium European Economic Interest Grouping
WHO	World Health Organization
Zn	zinc

Appendix A – List of Risk Assessment Reports on manganese and manganese compounds

Besides the reports cited in the Background section and in the text as references, risk assessments from other EU bodies and Institutions have been carried out.

1. EC Health and Consumers Scientific Committees Opinions

Scientific Committee on Food. Opinion on arsenic, barium, fluoride, boron and manganese in natural mineral waters (Expressed on 13 December 1996)

(http://ec.europa.eu/food/fs/sc/oldcomm7/out09_en.html)

2. European and other countries Risk Assessment Reports

Food Standard Agency Risk Assessment Manganese

(http://www.food.gov.uk/multimedia/pdfs/evm_manganese.pdf)

3. EFSA ANS Panel Opinions

Manganese ascorbate, manganese aspartate, manganese bisglycinate and manganese pidolate as sources of manganese added for nutritional purposes to food supplements - Scientific Opinion of the Panel on Food Additives and Nutrient Sources added to Food (ANS).

(http://www.efsa.europa.eu/en/efsajournal/doc/1114.pdf)

4. EFSA CEF Panel Opinions

Scientific Report of EFSA on the risk assessment of salts of authorised acids, phenols or alcohols for use in food contact materials.

(http://www.efsa.europa.eu/en/efsajournal/doc/1364.pdf)

5. EFSA NDA Panel Opinions

Scientific Report submitted to EFSA - Literature search and review related to specific preparatory work in the establishment of Dietary Reference Values: Preparation of an evidence report identifying health outcomes upon which Dietary Reference Values could potentially be based for chromium, manganese and molybdenum. (http://www.efsa.europa.eu/en/efsajournal/doc/1147.pdf)

Scientific Opinion on the substantiation of health claims related to manganese and protection of DNA, proteins and lipids from oxidative damage (ID 309), maintenance of bone (ID 310), energy-yielding metabolism (ID 311), and cognitive function (ID 340) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. (http://www.efsa.europa.eu/en/efsajournal/doc/1217.pdf)

Scientific Opinion on the substantiation of health claims related to manganese and reduction of tiredness and fatigue (ID 312), contribution to normal formation of connective tissue (ID 404) and contribution to normal energy-yielding metabolism (ID 405) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. (http://www.efsa.europa.eu/en/efsajournal/doc/1808.pdf)

Appendix B – List of authorisations of manganese compounds other than as feed additives

The following manganese compounds are authorised for use in food (Regulation (EC) No 1170/2009):[58] manganese ascorbate, manganese L-aspartate, manganese bisglycinate, manganese carbonate, manganese chloride, manganese citrate, manganese gluconate, manganese glycerophosphate, manganese pidolate and manganese sulphate which may be used in the manufacture of food supplements; manganese carbonate, manganese chloride, manganese citrate, manganese gluconate, manganese glycerophosphate and manganese sulphate which may be used in the manufacture of food supplements and may be added to food.

The following manganese compounds can be used for the manufacturing of dietetic foods (Commission Regulation (EC) No 953/2009):[59] manganese carbonate, manganese chloride, manganese citrate, manganese gluconate, manganese glycerophosphate and manganese sulphate.

The following manganese compounds can be used for the manufacturing of processed cereal-based foods and baby foods for infants and young children (Commission Directive 2006/125/EC):[60] manganese carbonate, manganese chloride, manganese citrate, manganese gluconate, manganese glycerophosphate and manganese sulphate.

Regarding pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin, the following manganese compounds are listed in Table 1 of the Annex of Regulation 37/2010[61] as *Allowed substances, no MRL required*: dimanganese trioxide, manganese carbonate, manganese chloride, manganese gluconate, manganese glycerophosphate, manganese oxide, manganese pidolate, manganese ribonucleate and manganese sulphate. According to article 14(7) of Regulation (EC) No 470/2009[62] these compounds are for oral use only.

The following manganese compound is listed in Annex of Commission Implementing Regulation (EU) No 540/2011[63] as "Active substances approved for use in plant protection products": manganese ethylenebis (dithiocarbamate, polymeric) (Maneb) and manganese ethylenebis (dithiocarbamate, polymeric) complex with zinc salt (Mancozeb).

The following type of fertilisers for manganese as *Fertilisers containing only one micro-nutrient* are listed in Annex I of Regulation (EC) No 2003/2003[64] of the European Parliament and of the Council: (a) manganese salt (chemically obtained product containing a mineral manganese salt (Mn II) as its essential ingredient), (b) manganese chelate (water-soluble product obtained by combining manganese chemically with a chelating agent), (c) manganese oxide (chemically obtained product containing manganese oxides as essential ingredients), (d) manganese-based fertiliser (product obtained by mixing types (a) and (c)) and (e) manganese-based fertiliser solution (product obtained by dissolving types (a) and/or one of the type (b) in water).

[58] 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.

[59] 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.

[60] 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.

[61] 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.

[62] Regulation (EC) no 470/2009 of the European Parliament and of the Council of 6 May 2009 laying down Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin, repealing Council Regulation (EEC) No 2377/90 and amending Directive 2001/82/EC of the European Parliament and of the Council and Regulation (EC) No 726/2004 of the European Parliament and of the Council OJ L 152, 16.6.2009, p. 11.

[63] 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.

[64] 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.

The following manganese compounds can be used for cosmetic purposes (Regulation (EC) No 1223/2009 of the European Parliament and of the Council):[65] ammonium manganese (3+) diphosphate and trimanganese bis (orthophosphate).

According to the Annex of Regulation (EC) No 432/2012[66] the following health claims can be made only for food which is at least a source of manganese 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[67]: manganese contributes to normal energy-yielding metabolism, manganese contributes to the maintenance of normal bones, manganese contributes to the normal formation of connective tissue and manganese contributes to the protection of cells from oxidative stress.

[65] 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.
[66] 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.
[67] 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 C – Details on the Characterisation and Identity of the compounds for which more than one company is involved in the application[68]

Table 3: Parameters of characterisation and identity of manganous oxide (number of batches in brackets)

	Company[(a)]	
	b5	**b7**
Manganese content (%)	61.7–62.7 (5)[(1)]	60.3–61.1 (5)[(2)]
Pb (mg/kg)	154–165 (5)[(3)]	71.4–180 (5)[(4)]
Cd (mg/kg)	6–7 (5)	5.2–8.2 (5)
Hg (mg/kg)	0.02–0.06 (3)[(5)]	0.45–0.68 (5) < 0.02–0.04 (22)[(6)]
F (mg/kg)	---	90 (1)
As (mg/kg)	43 (5)	34.1–44 (5)
Dioxins[(b)]	0.035–0.094 (4)[(7)]	0.039–0.090 (2)[(8)]
Sum of dioxins and dioxin-like PCBs[(c)]	0.090–0.064 (2)[(9)]	0.071–0.121 (2)
Nickel (mg/kg)[(10)]	370–400 (3)	681–867 (2)
Particle size (%)[(d)]	30.4 (< 50.2 µm) 13.7 (< 10.0 µm) (1)[(11)]	41.5 (< 52.6 µm) 23 (< 10.8 µm) (1)[(12)]
Dusting potential (g/m^3)	---	4.22[(13)]

--- : Not provided.
(1): Technical Dossier/Section II/Annex 2-1-23 and 2-1-41
(2): Technical Dossier/Section II/Annex 2-1-24
(3): Technical Dossier/Section II/Annex 2-1-41 and 2-1-23 (For heavy metals (Cd and Pb) and As)
(4): Technical Dossier/Section II/Annex 2-1-42 and 2-1-24 (For heavy metals (Cd, Hg and Pb) and As)
(5): Technical Dossier/Supplementary Information (April 2015)/Annex_Qxii_Mn oxide_Mercury.pdf mercury(2)pdf. and mercury(3)pdf
(6): Technical Dossier/ Supplementary Information (April 2015)/Annex_Qxiii_Mn oxide_mercury_Company b7(1)-(22).pdf
(7): Technical Dossier/Section II/Annex 2-1-59. Technical Dossier/Supplementary Information (April 2015)/Annex_Qxii_Mn oxide_Dioxins and Dioxin like PCBs.pdf
(8): Technical Dossier/Section II/Annex 2-1-60
(9): Technical Dossier/Supplementary Information (April 2015)/Annex_Qxii_Mn oxide_Dioxins and Dioxin like PCBs.pdf
(10):Technical Dossier/Supplementary Information (April 2015)/Annex_Qii_Nickel_Mn oxide.pdf
(11):Technical Dossier/Section II/Annex 2-2-31
(12):Technical Dossier/Section II/Annex 2-2-32 (the compound measured is not identified)
(13):Technical Dossier/Supplementary Information (April 2015)/Annex_Qxiv_Mn oxide_Dusting potential.pdf

(a): Two companies: b5 and b7
(b):Expressed as ng WHO-PCDD/F-TEQ/kg
(c):Expressed as ng WHO-PCDD/F- PCB-TEQ/kg)
(d):Technique used. Laser diffraction (expressed as v/v)

[68] This section has been amended following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.

Table 4: Parameters of characterisation and identity of manganous sulphate, monohydrate (number of batches in brackets).

	Company[a]	
	b7	**b12**
Manganese content (%)	31.4–32.1 (4)[1]	32.0–34.1(6)[2]
Pb (mg/kg)	< 0.5–33 (4)[3]	<0.5–9(6)[4]
Cd (mg/kg)	0.91–16 (4)	0.57–5 (6)
Hg (mg/kg)	< 0.01–< 0.02(4)	<0.02–0.2 (6)
F (mg/kg)	< 70 (1)	---
As (mg/kg)	< 0.5–9.7 (4)	0.08–0.19 (6)
Dioxins[b]	0.047 –0.09 (2)[5]	0.09 (2)[6]
Sum of dioxins and dioxin-like PCBs[c]	0.073–0.119 (4)	0.137–0.138 (2)
Nickel (mg/kg)[7]	516–530 (2)	23–891 (5)
Particle size (%)[d]	6.5 (< 52.6 µm) 1.6 (< 10.8 µm) (1)[8]	0 (< 50 µm) 0 (< 10 µm) (1)[9]
Dusting potential (g/m^3)	6.89 (1)[10]	---

--- : Not provided
(1): Technical Dossier/Section II/Annex 2-1-25
(2): Technical Dossier/Section II/Annex 2-1-26 and 2-1-44 and 2-1-62
(3): Technical Dossier/Section II/Annex 2-1-43 and 2-1-25 (For heavy metals (Cd, Hg and Pb) and As)
(4): Technical Dossier/Section II/Annex 2-1-44 and 2-1-26 (For heavy metals (Cd, Hg and Pb) and As)
(5): Technical Dossier/Section II/Annexes 2-1-61and 2-1-25 (Dioxin and Sum of dioxin and Dioxin like)
(6): Technical Dossier/Section II/Annexes 2-1-62 (Dioxin and Sum of dioxin and Dioxin like)
(7): Technical Dossier/Supplementary Information (April 2015)/Annex_Qii_Nickel_Mn sulphate mono.pdf
(8): Technical Dossier/Section II/Annex 2-2-33
(9): Technical Dossier/Section II/Annex 2-2-34
(10): Technical Dossier/Supplementary Information (April 2015)/Annex_Qxi_Mn sulphate H2O_Dusting potential.pdf

(a): Two companies: b7 and b12
(b): Expressed as ng WHO-PCDD/F-TEQ/kg
(c): Expressed as ng WHO-PCDD/F- PCB-TEQ/kg)
(d): Technique used. Laser diffraction (expressed as v/v)

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

	Company[a]				
	b1	**b2**	**b4**	**b9**	**b12**
Manganese content (%)	10.2–10.6 (5)[1]	15.2–15.9 (7)[2]	16.1–16.7 (5)[3]	12.2–13.2 (3)[4]	15.1–15.6 (5)[5]
Pb (mg/kg)	< 0.05–<0.1 (3)[6]	6–20 (7)[7]	< 0.5 (1)[8]	< 0.5–0.31 (3)[9]	0.1–0.8 (3)[10]
Cd (mg/kg)	0.6–1.2 (3)	1–7(7)	< 0.5 (1)	< 0.1–<0.5 (3)	0.8–1.8 (3)
Hg (mg/kg)	< 0.005(3)	---	< 0.02 (1)	< 0.1–<0.5(3)	< 0.01(3)
As (mg/kg)	< 0.10–0.25 (3)	1 (1)	< 0.5 (1)	< 0.5–0.58 (3)	0.2–0.3 (3)
F (mg/kg)	< 10 (3)	---	---	---	---
Dioxins[b]	0.050–0.148 (3)[11]	0.055 (1)[12]	0.09 (1)[13]	0.015 (1)[14]	0.09–0.16 (4)[15]
Sum of dioxins and dioxin-like PCBs[c]	0.079–0.186 (3)	---	0.123 (1)	0.016 (1)	0.318 (1)
Nickel (mg/kg)[16]	141 (1)	20 (1)	144–472 (2)	175–178 (2)	11 (1)
Particle size (%)[D]	59.8–73.0 (< 52.9 µm) 19.5–22.5 (< 10 µm) (4)[17]	50.8 (< 50 µm) 12.6 (< 10 µm) (1)[18]	13.8 (< 63 µm) --- (1)[19]	3.9–4.3 (< 50 µm) 0 (< 10 µm) (3)[20]	59.6 < 52.3 µm 3.1 < 11 µm (1)[21]
Dusting potential (g/m^3)	0.15–0.88 (5)[22,23]	---	---	---	---

--- : Not provided

(1): Technical Dossier/Section II/Annex 2-1-10
(2): Technical Dossier/Section II/Annex 2-1-11and 2-1-29
(3): Technical Dossier/Section II/Annex 2-1-13
(4): Technical Dossier/Section II/Annex 2-1-15
(5): Technical Dossier/Section II/Annex 2-1-16
(6): Technical Dossier/Section II/Annex 2-1-28 (For heavy metals (Cd, Hg and Pb) and As)
(7): Technical Dossier/Section II/Annex 2-1-29 and 2.1.11(For heavy metals (Cd, Hg and Pb) and As)
(8): Technical Dossier/Section II/Annex 2-1-31 and 2-1-49 (For heavy metals (Cd, Hg and Pb) and As)
(9): Technical Dossier/Section II/Annex 2-1-33 and 2-1-15 (For heavy metals (Cd, Hg and Pb) and As)
(10): Technical Dossier/Section II/Annex 2-1-34 and 2-1-52 (For heavy metals (Cd, Hg and Pb) and As)
(11): Technical Dossier/Section II/Annex 2-1-46 (Dioxin and Sum of dioxin and Dioxin like)
(12): Technical Dossier/Section II/Annex 2-1-47 (Dioxin and Sum of dioxin and Dioxin like)
(13): Technical Dossier/Section II/Annex 2-1-49 and 2-1-31(Dioxin and Sum of dioxin and Dioxin like)
(14): Technical Dossier/Section II/Annex 2-1-51 (Dioxin and Sum of dioxin and Dioxin like)
(15): Technical Dossier/Section II/Annex 2-1-52 and 2-1-34 (Dioxin and Sum of dioxin and Dioxin like). Technical Dossier/Supplementary Information (April 2015)/Annex_Qxv_Mn AA chelate_Company b12_Dioxins and Dioxin like PCBs.pdf
(16): Technical Dossier/Supplementary Information (April 2015)/Annex_Qii_Nickel_Mn AA.pdf
(17): Technical Dossier/Section II/Annex 2-2-18
(18): Technical Dossier/Section II/Annex 2-2-19
(19): Technical Dossier/Section II/Annex 2-2-21
(20): Technical Dossier/Section II/Annex 2-2-23
(21): Technical Dossier/Section II/Annex 2-2-24
(22): Technical Dossier/Supplementary Information (April 2015)/Annex_Qxvi_Mn chelate AA_Dusting potential.pdf
(23): Technical Dossier/Supplementary Information (April 2015)/Annex_Qxvi_Mn AA chelate_Mn in dust.pdf

(a): Five companies: b1, b2, b4, b9 and b12
(b): Expressed as ng WHO-PCDD/F-TEQ/kg
(c): Expressed as ng WHO-PCDD/F- PCB-TEQ/kg
(d): Technique used. b1, b2, b9 and b12 Laser diffraction (expressed as v/v). b4 Sieving (expressed as w/w)

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

Company[a] (number of batches)	Production date Date of analysis	Age (months)	Contaminants	CFU/g
b1 (1)[1]	March 2010	36	Total aerobic plate count	$< 1.0 \times 10^{(2)}$
			Total Yeasts and Moulds	$< 1.0 \times 10^{(1)}$
			Coliforms	$< 1.0 \times 10^{(2)}$
	April 2013		*Salmonella*	Negative in 25 g
b2 (1)[2]	October2010	ca. 27	Total plate count	$< 1.0 \times 10^{(1)}$
			Total Yeasts and Moulds	Negative in 0.1g
	January 2013		*E. coli*	$< 1.0 \times 10^{(1)}$
			Salmonella	Negative in 25 g
b4 (1)[3]	January 2009	35	Yeasts	$< 1.0 \times 10^{(2)}$
			Moulds	$< 1.0 \times 10^{(2)}$
	December 2012		*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	$< 1.0 \times 10^{(2)}$
b9 (1)[4]	February 2009	ca. 37	Yeasts	$< 2.0 \times 10^{(1)}$
			Moulds	$< 2.0 \times 10^{(1)}$
	March 2012		*Salmonella*	Negative in 25 g
			Total bacterial count - aerobic	$< 0.5 \times 10^{(1)}$
			Total bacterial count –	$< 0.5 \times 10^{(1)}$
			anaerobic	$< 2.0 \times 10^{(1)}$
			Total coliforms	(NPP/g)
b12[5] (1)	Sept./Oct. 2012	1-3	Yeasts	$< 1.0 \times 10^{(2)}$
			Moulds	$< 1.0 \times 10^{(2)}$
	Nov./Dec. 2012		*Salmonella*	Negative in 25g
			Total plate count	$< 1.0 \times 10^{(3)}$
			Total Coliforms	$< 1.0 \times 10^{(1)}$
(2)	July 2009/Sept. 2009	38-40	Yeasts	$< 1.0 \times 10^{(2)}$
			Moulds	$< 1.0 \times 10^{(2)}$
	November 2012		*Salmonella*	Negative in 25g
			Total plate count - aerobic	$< 1.0 \times 10^{(3)}$
			Total Coliforms	$< 1.0 \times 10^{(1)}$

(1): Technical Dossier/Supplementary Information (April 2015)/Annex_Qxix_Mn chelate AA_mycotoxins and microbio_Company 1.pdf 2-1-62
(2): Technical Dossier/Supplementary Information (April 2015)/Annex_Qxix_Mn chelate AA_microbio_Company b2.pdf Expressed as ng WHO-PCDD/F-TEQ/kg
(3): Technical Dossier/Supplementary Information (April 2015)/Annex_Qxix_Mn chelate AA_microbio_Company b4.pdf
(4): Technical Dossier/Supplementary Information (April 2015)/Annex_Qxix_Mn chelate AA_ microbio_Company b9.pdf
(5): Technical Dossier/Supplementary Information (April 2015)/Annex_Qxix_Mn chelate AA_mycotoxins and microbio_Company b12.pdf
(a): Five companies: b1, b2, b4, b9 and b12

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

	Company[a]				
	b2		b4	b9	b11
	Higher grade	Lower grade[1]			
Manganese content (%)	22.1–23.4 (6)[2]	15.8–16.0 (5)	21.8–22.1 (5)[3]	22–23 (3)[4]	26.9–27 (5)[5]
Pb (mg/kg)	8–10 (6)[6]	8–14 (5)	<0.5(3)[7]	<0.5 (3)[8]	33.7–34 (3)[9]
Cd (mg/kg)	1–9 (6)	1–4 (5)	0.79–2.21 (3)	<0.5–4 (3)	2.21–2.34 (3)
Hg (mg/kg)	---	---	<0.02 (3)	<0.5 (3)	<0.0007(3)
As (mg/kg)	1 (1)	1 (5)	<0.5 (3)	<0.5–0.6 (3)	<0.01 (3)
Dioxins[b]	0.065 (1)[10]	0.043–0.067 (5)	0.09 (2)[11]	0.17 (1)[12]	0.0184 (1)[13]
Sum of dioxins and dioxin-like PCBs[c]	---	0.053–0.077 (5)	0.126–0.153	---	0.0298
Nickel (mg/kg)[14]	53	---	178–501	475	96
Particle size (%)[d]	100 (< 50 µm) 93.5 (< 10 µm) (1)[15]	---	10.9 (<52.62 µm) 4.2 (<10.78 µm) (1)[16]	8.8–9.3 (< 50 µm) 0.8–0.9 (< 10 µm) (3)[17]	5* (< 50 µm) 2.5 (< 10 µm) (1)[18]
Dusting potential (g/m^3)	2.23[19]	---	---	---	---

--- : Not provided

(1): Technical Dossier/Supplementary Information (April 2015)/Annex_Company b2_Composition_Mn chelate glycine_15%_5CoAs.pdf

(2): Technical Dossier/Section II/Annex 2-1-17 and 2-1-35

(3): Technical Dossier/Section II/Annex 2-1-18

(4): Technical Dossier/Section II/Annex 2-1-19

(5): Technical Dossier/Section II/Annex 2-1-20

(6): Technical Dossier/Section II/Annex 2-1-35 and 2-1-17 (For heavy metals (Cd, Hg and Pb) and As)

(7): Technical Dossier/Section II/Annex 2-1-36 and 2-1-54 (For heavy metals (Cd, Hg and Pb) and As)

(8): Technical Dossier/Section II/Annex 2-1-19 (For heavy metals (Cd, Hg and Pb) and As)

(9): Technical Dossier/Section II/Annex 2-1-38 (For heavy metals (Cd, Hg and Pb) and As)

(10): Technical Dossier/Section II/Annex 2-1-53 (Dioxin and Sum of dioxin and Dioxin like)

(11): Technical Dossier/Section II/Annex 2-1-54 and 2-1-36 (Dioxin and Sum of dioxin and Dioxin like)

(12): Technical Dossier/Section II/Annex 2-1-55 (Dioxin and Sum of dioxin and Dioxin like)

(13): Technical Dossier/Section II/Annex 2-1-56 (Dioxin and Sum of dioxin and Dioxin like)

(14): Technical Dossier/Supplementary Information (April 2015)/Annex_Qii_Nickel_Mn glycinate.pdf

(15): Technical Dossier/Section II/Annex 2-2-25

(16): Technical Dossier/Section II/Annex 2-2-26

(17): Technical Dossier/Section II/Annex 2-2-27

(18): Technical Dossier/Section II/Annex 2-2-28

(19): Applicant B. Technical Dossier/Supplementary Information (April 2015)/Annex_Qxxiii_Mn chelate glycine_Dusting potential.pdf

(a): Four companies: b2, b4, b9 and b11

(b): Expressed as ng WHO-PCDD/F-TEQ/kg

(c): Expressed as ng WHO-PCDD/F-PCB-TEQ/kg

(d): Technique used. Laser diffraction (expressed as v/v). (*) Extrapolated from the graph

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

	Company[a]		
	b2-Lower grade[1]	**b4[2]**	**b9[3]**
Manganese Content (%)	16.2–16.3 (3)	19.5–20.3 (3)	22.4–22.7 (3)
Extractable glycine (%)	49.9–51.0 (3)	40.6–41.7 (3)	32.2–32.9 (3)
Sulphur (%)	9.5–9.9 (3)	12.5–15.2 (3)	14.2–14.4 (3)
Sulphate (%)	28.5–29.6 (3)	37.5–37.8 (3)	42.5–42.4 (3)
Moisture (%)	3.2–5.2 (3)	0.4–0.5 (3)	1.7–1.9 (3)

(1): Technical Dossier/ Supplementary Information (April 2015)/ MnGly_Composition_Company b2.pdf
(2): Technical Dossier/ Supplementary Information (April 2015)/ MnGly_Composition_Company b4.pdf
(3): Technical Dossier/ Supplementary Information (April 2015)/ MnGly_Composition_Company b9.pdf

(a): Three companies: b2, b4 and b9

Appendix D – Calculation of maximum manganese content in water for drinking in selected animal species

The manganese background of complete feed for monogastric and TMR for ruminants varies generally between 20 and 80 mg Mn/kg, depending on the composition of the feed (Jeroch et al., 1993; van Paemel et al., 2010). For further calculations, 50 mg Mn/kg DM are considered as background level of complete feed (or of TMR) and 10 mg/kg milk replacer. On the base of the EFSA Guidance on tolerance and efficacy studies (EFSA FEEDAP Panel, 2011), the FEEDAP Panel derived the following ratios of water to feed intake: 2 for poultry, 3 for pigs and 8 for milk replacer (assuming that the water used for preparing the liquid feed is water for drinking). Applying these values, the following maximum concentrations were calculated: 50 mg Mn/L for poultry; 33 mg Mn/L for pigs and 18 mg Mn/L for calves. Furthermore, the EFSA Guidance (EFSA FEEDAP Panel, 2011) notes that for ruminants, concentrations for an additive in water for drinking cannot be consistently extrapolated from a fixed ratio of feed to water intake; e.g. the water intake of dairy cattle depends on milk yield, ambient temperature, body weight, feed dry matter content, sodium content of ration and further factors (Castle and Thomas, 1975; Murphy et al., 1983; Holter and Urban, 1992; Dahlborn et al., 1998; Meyer et al., 2004) and may vary between 90 and 140 L/day for a cow with 650 kg body weight and producing 30 L milk/day (Meyer et al., 2004; Ward and McKague, 2007). Under consideration of a water intake of 8L/100 kg bw and day (Meyer et al. 2006; Ward and McKague 2007), 30 mg Mn can be supplemented/L water.

The maximum total daily manganese intake of dairy cows was calculated to be 3405 mg Mn/day based on the following assumptions: 650 kg body weight, feed intake of 22.7 kg (complete feed containing 88% dry matter= 20 kg DM/day (DM)) and diets contain the maximum authorised content (150 mg Mn/kg complete feed). The maximum manganese quantity in water for drinking is given by the difference between the maximum dietary manganese intake (3405 mg/day) and the manganese background already provided by feed materials. An expected background concentration in dairy feed (TMR) of 50 mg Mn/kg DM would correspond to a total intake of 1000 mg/day. Therefore, assuming this manganese background concentration, the maximum quantity of manganese that could be provided daily by water for drinking is 2405 (3405 – 1000) mg Mn/day; this daily dose complies with the maximum amount set for complete feed, independent from its dilution in water for drinking, which will obviously depend on water intake. For instance, for a cow with low or high water intake, about 27 (at 90 L per cow and day) or 17 mg Mn/L water for drinking (at 140 L per cow and day) could be supplemented. The same approach can be applied for other ruminant categories, e.g. for beef cattle (400 kg bw, 9.09 kg feed/day= 8 kg feed DM) resulted in 960 (1364 – 400) mg Mn/day (240 mg Mn/100 kg bw) via water for drinking.

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Appendix E – Manganese deposition in liver and muscle

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

Animal	Manganese (mg/kg feed)		Deposition in liver (mg Mn/kg liver)		Deposition in muscle (mg Mn/kg muscle)		Deposition in egg (mg Mn/kg egg yolk)		Reference
	Total	Suppl.	Inorg.	Organic	Inorg.	Organic	Inorg.	Organic	
Broiler									
$MnSO_4$/ Mn-Prot	n.d.	80	4.8	5.2	-	-	-	-	Bao et al., 2007
Layer									
MnO/ Mn-AA	54.7/84.7	30/60	-	-	-	-	0.79/0.93	0.72/0.94	Mabe et al., 2003
Piglet									
$MnSO_4$/ Mn-Prot	137 (inorg.) 140 (org.)	100	0.34	0.33	-	-	-	-	Schiavon et al., 2000
$MnSO_4$/ Mn-Prot	19	4	3.41	3.71	0.05	0.14	-	-	Martin et al., 2011
Dairy Cow									
$MnSO_4$/ Mn-AA	46	18	9.4	9.6	-	-	-	-	Nocek et al., 2006
$MnSO_4$/ Mn-AA	n.d.	30	11	12	-	-	-	-	Olson et al., 1999
Rainbow Trout									
$MnSO_4$/ Mn-AA	14	n.d.	-	-	2.4	2.7	-	-	Apines et al., 2003
$MnSO_4$/ Mn-AA	37	20	-	-	1.58	1.22	-	-	Apines et al., 2004

AA: Aminoacid; Prot: protein or hydrolysed protein; n.d.: not described

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Annex – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Manganese (E5)

In the current application authorisation is sought under articles 4(1) and 10(2) for manganese chelate of glycine hydrate[1], manganese chelate of amino acids hydrate[1,2], manganous oxide[1,3], manganous carbonate[1], manganous chloride tetrahydrate[1] and manganous sulphate monohydrate[1,3] under the category/ functional group (3b) 'nutritional additives'/'compounds of trace elements', according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of these feed additives for all categories and species.

According to the Applicants *manganese chelate of glycine hydrate* is a beige to pink free-flowing powder with a minimum content of 20% *total manganese*, *manganese chelate of amino acids hydrate* is a off-white to tan marbeled free-flowing powder with a minimum content of 10% *total manganese*, *manganous oxide* is a red brown-green powder with a minimum content of 59.5% *total manganese*, *manganous carbonate* is a beige powder with a minimum content of 44% *total manganese*, *manganous chloride tetrahydrate* is a pink powder with a minimum content of 27% *total manganese* and *manganous sulphate monohydrate* is a pink-grey crystalline powder with a minimum content of 31% *total manganese*. These *feed additives* are intended to be mixed into *premixtures, feedingstuffs* and *water*.* The Applicants suggested maximum levels ranging from 100 to 150 mg *total manganese* /kg *feedingstuffs* and from 15 to 75 mg *total manganese* /L *water*, similar to limits set in the previous regulations [4,5].

For the characterisation of *manganous sulphate monohydrate* in the *feed additive* the EURL recommends the titrimetric method described in the European Pharmacopoeia monograph 1543.

For the quantification of 'amino acid' content in the amino manganese chelates (i.e. *manganese chelate of glycine hydrate* and *manganese chelate amino acids hydrate*), the Applicant (FAD-2010-0088) proposed the Community method based on ion exchange chromatography combined with post-column ninhydrin derivatisation 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.

Furthermore, the EURL identified the generic European Pharmacopoeia methods for the 'identification reactions of ions and functional groups', such as carbonate, chloride and sulphate in the *feed additives*. Finally, the EURL recommends crystallographic techniques, such as X-Ray diffraction for the characterisation of crystalline structures of *manganous oxide, manganous chloride tetrahydrate, manganous carbonate* and *manganous sulphate monohydrate*.

For the quantification of total manganese in the feed additives, premixtures and feedingstuffs the Applicants submitted three ring trial validated CEN methods: EN 6869, based on atomic absorption spectrometry (AAS), EN 15510, based on inductively coupled plasma atomic emission spectroscopy (ICP-AES) and CEN/TS 15621, based on ICP-AES after pressure digestion. Precisions ranging from 2 to 20% were reported, together with

limits of quantification (LOQ) ranging from 1 to 5 mg/kg feedingstuffs. Furthermore, the EURL identified the comparative trial organised by the UK Food Standards Agency, based on the Community method for the determination of manganese in feedingstuffs, in which precisions ranging from 2.7 to 7.1% were reported.

For the quantification of total manganese in water the EURL identified the ring trial validated method EN ISO 11885, based on ICP-AES. The following performance characteristics are reported: - a relative standard deviation for repeatability (RSDr) ranging from 1.3 to 1.8%; - a relative standard deviation for reproducibility (RSDR) ranging from 4.6 to 6.0%; and LOQ = 1 µg/L.

[1] FAD-2010-0088
[2] FAD-2010-0069
[3] FAD-2010-0235

Based on the available performance characteristics the EURL recommends for official control all the above mentioned CEN methods together with the Community method to quantify total manganese content in the feed additives, premixtures, feedingstuffs and/or 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.

(*) *for manganese chelate of glycine hydrate, manganous chloride tetrahydrate and manganous sulphate monohydrate* (FAD-2010-0088).