SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of manganese compounds (E5) as feed additives for all species: manganous oxide and manganous sulphate monohydrate, based on a dossier submitted by Eramet & Comilog Chemicals S.A.[1]

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

European Food Safety Authority (EFSA), Parma, Italy

This scientific output, published on 19 March 2015, replaces the earlier version published on 30 October 2013.[4]

ABSTRACT

Manganese, an essential trace element, functions as an enzyme activator and is a constituent of several enzymes. Primary signs of manganese deficiency are impaired growth, skeletal abnormalities, depressed reproductive function, ataxia of the newborn and faults in lipid and carbohydrate metabolism. Manganous oxide and manganous sulphate monohydrate are safe sources of manganese for all animal species/categories, provided that the current maximum total contents of manganese authorised in feed are respected. Generally, dietary manganese does not appear to cause any adverse health effects in the population and has not proven to be a risk at the usual intake levels. Manganese intake resulting from the consumption of tissues and products of animal origin is low and not of concern for the safety of consumers, including more sensitive subgroups such as infants and elderly people. It is concluded that the use of manganous oxide and manganous sulphate monohydrate 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. The handling of manganous oxide and manganous sulphate monohydrate poses a risk to users upon inhalation exposure. The additive manganous oxide should be considered as a potential skin and eye irritant and as a dermal sensitiser. Manganous sulphate monohydrate is not irritating to skin but it is an eye irritant; it is likely not a dermal sensitiser. The use of manganous oxide and manganous sulphate monohydrate in animal nutrition for all animal species is not of concern for the environment, provided that the current maximum total contents of manganese authorised in feed are respected. Manganous oxide and manganous sulphate monohydrate are efficacious sources of manganese in meeting animals' requirements.

[1] On request from the European Commission, Question No EFSA-Q-2012-00438, adopted on 9 October 2013.

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

[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Trace Elements, including Noël Albert Dierick, Jürgen Gropp and Alberto Mantovani, for the preparatory work on this scientific opinion.

[4] This scientific opinion has been amended following the adoption of the decision of the Commission on confidentiality claims submitted by the applicant, in accordance with Article 8(6) and Article 18 of Regulation (EC) No 1831/2003. The modified sections are indicated in the text. To avoid confusion, the original version has been removed from the EFSA Journal, but is available on request, as is a version showing all the changes made.

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KEY WORDS

nutritional additives, manganese, manganous sulphate mohohydrate, manganous oxide, safety, environment, efficacy

SUMMARY

Following a request from European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of manganous oxide and manganous sulphate monohydrate when used as feed additives for all animal species.

Manganese, an essential trace element, functions as an enzyme activator and is a constituent of several enzymes (e.g. glycosyl transferases, pyruvate carboxylase, manganese superoxide dismutase). Primary signs of manganese deficiency are impaired growth, skeletal abnormalities, depressed reproductive function, ataxia of the newborn and faults in lipid and carbohydrate metabolism.

Manganous oxide and manganous sulphate monohydrate are considered safe for all animal species/categories, provided that the maximum total contents of manganese authorised in feed (100 mg/kg complete feedingstuffs for fish and 150 mg/kg for other species) are respected.

Generally, dietary manganese does not appear to cause any adverse health effects in the population and has not proven to be a risk at the usual intake levels. Manganese intake resulting from the consumption of tissues and products of animal origin is low and not of concern for the safety of consumers, including more sensitive subgroups such as infants and elderly people. It is concluded that the use of manganous oxide and manganous sulphate monohydrate 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.

The handling of manganous oxide and manganous sulphate monohydrate poses a risk to users upon inhalation exposure. The additive manganous oxide should be considered as a potential skin and eye irritant and as a dermal sensitiser. Manganous sulphate monohydrate is not irritating to skin but it is an eye irritant; it is likely not a dermal sensitiser.

The use of manganous oxide and manganous sulphate monohydrate in animal nutrition for all animal species is not of concern for the environment, provided that the current maximum total contents of manganese authorised in feed are respected.

Manganous oxide and manganous sulphate monohydrate are efficacious sources of manganese in meeting animals' requirements.

The FEEDAP Panel made some specific recommendations concerning the description and conditions of use of the additive in the registry entry, including a recommendation related to the contribution of cobalt from the additive manganous oxide to diets.

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BACKGROUND

Regulation (EC) No 1831/2003[5] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 10(2) of that Regulation specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, at the latest one year before the expiry date of the authorisation given pursuant to Directive 70/524/EEC for additives with a limited authorisation period, and within a maximum of seven years after the entry into force of this Regulation for additives authorised without a time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from ERAMET & Comilog Chemicals S.A.[6] for re-evaluation of authorisation of the manganese-containing additives manganous oxide (Alma®) and manganous sulphate monohydrate, when used as a feed additive for all animal species (category: Nutritional additives; functional group: compound of trace elements) under the conditions mentioned in Table 1.

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 10(2) (re-evaluation of an authorised feed additive). EFSA received directly from the applicant the technical dossier in support of this application.[7] According to Article 8 of that Regulation, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. The particulars and documents in support of the application were considered valid by EFSA as of 25 of September of 2012.

The additives 'Manganous oxide' and 'Manganous sulphate monohydrate' had been authorised in the EU under the element Manganese-Mn for all animal species 'Without a time limit' (Commission Regulation (EC) No 1334/2003[8] and amendments, and Commission Regulation (EC) No 479/2006).[9] 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'.[10]

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), three opinions on a manganese chelate of hydroxy analogue of methionine (EFSA, 2008a; EFSA FEEDAP Panel, 2009a, 2010), and has recently delivered two opinions on re-evaluation of manganese compounds (manganese chelate of amino acids, hydrate (EFSA FEEDAP Panel, 2013a) and manganous oxide (EFSA FEEDAP Panel, 2013b).

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall determine whether the feed additives comply with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and the efficacy of the manganous oxide and manganous sulphate monohydrate when used under the conditions described in Table 1.

[5] 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.
[6] ERAMET & Comilog Chemicals S.A., Rue du bois, 7334-ST Ghislain, Belgium.
[7] EFSA Dossier reference: FAD-2010-0166.
[8] 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.
[9] 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.
[10] European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003. Available online: http://ec.europa.eu/food/food/animalnutrition/feedadditives/comm_register_feed_additives_1831-03.pdf

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

Additive	Manganese: MnO - $MnSO_4.H_2O$
Registration number/EC No/No (if appropriate)	E 5
Category(-ies) of additive	Nutritional additives
Functional group(s) of additive	Compounds of trace elements

Description			
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)
MnO	**MnO**	**Difference between Mn total and Mn soluble HCl max. 1%**	**MnTotal: ISO 4298:1994 Mn soluble HCl: potentiometric method**

Trade name (if appropriate)	MnO Alma®
Name of the holder of authorisation (if appropriate)	-

Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period (if appropriate)
		mg/kg of complete feedingstuffs		
All species			150	
Fish			100	

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use (if appropriate)	
Specific conditions or restrictions for handling (if appropriate)	See MSDS
Post-market monitoring (if appropriate)	
Specific conditions for use in complementary feedingstuffs (if appropriate)	

Maximum Residue Limit (MRL) (if appropriate)			
Marker residue	Species or category of animal	Target tissue(s) or food products	Maximum content in tissues

ASSESSMENT

This opinion is based in part on data provided by an applicant involved in the production/distribution of manganous oxide and manganous sulphate monohydrate. It should be recognised that these data cover only a fraction of those compounds existing on the market. The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) has sought to use the data provided together with data from other sources to deliver an opinion.

1. Introduction

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. Although the elemental (metal) form of manganese does not occur naturally in the environment, 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 mineralization, regulation of protein and energy metabolism, cellular protection from damaging free radical species and formation of glycosaminoglycans (Wedler, 1994). Manganese acts as both a constituent of metalloenzymes and as enzyme activator. Enzymes that contain manganese include arginase, pyruvate carboxylase and manganese-superoxide dismutase (NRC, 1989; Keen and Zidenberg-Cher, 1990; Wedler, 1994). Manganese, in its activating capacity, can bind either to a substrate (such as adenosine triphosphate, ATP) or to a protein directly, thereby causing conformational changes (Keen and Zidenberg-Cher,1990). Manganese has been shown to activate numerous enzymes involved with either a catalytic or regulatory function (e.g. transferases, decarboxylases, hydrolases) (Wedler, 1994).

The application is for the use of manganous oxide and manganous sulphate monohydrate in feed for all animal species/categories. These manganese compounds are already authorised in the European Union as nutritional additives.

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

EFSA commissioned the University of Gent (Belgium) to carry out a study of selected trace and ultratrace elements in animal nutrition, 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.

2. Characterisation[11]

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

2.1. Characterisation of the additives

2.1.1. Manganous oxide

'Manganous oxide' (Chemical Abstracts Service (CAS) No 1344-43-0; International Union of Pure and Applied Chemistry (IUPAC) name: Manganese(II) oxide) has the chemical formula MnO (molecular weight 70.94 g/mol; 77.39 % Mn). The product specification sheet indicates that the additive contains a minimum of 60 % manganese (without further specification of manganous oxide (MnO) content) and a maximum of 2 % manganese dioxide (MnO_2). The additive in the application is characterised to contain 79.8 % MnO (soluble in HCl) and, in lower proportion, aluminium oxide, iron oxide, silicium oxide and potassium oxide; the composition provided does not sum up to 100 %.

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

The manganese content of the additive measured in five batches confirmed the specification and corresponded to about 80 % manganous oxide. The same batches were analysed for iron, copper, cobalt, zinc and molybdenum.[12]

Impurities/contaminants (lead, cadmium, mercury, arsenic, dioxins and dioxin-like PCBs) were analysed on five batches of the additive. All values were below the maximum contents set in Directive 2002/32/EC, as well as below the action thresholds for dioxins and dioxins like-PCBs.[13] The concentrations of mercury do not give rise to safety concerns. The nickel content of the additive was analysed in ten batches; the applicant showed that the nickel monitoring is part of the implemented HACCP system.[14]

The product is a solid, green-coloured powder. It is insoluble in water. Bulk density is about 930 kg/m^3.

Data on particle size distribution by laser diffraction was provided (four batches).[15] The dusting potential was measured by the Stauber-Heubach method in two data sets of three batches.[16]

The applicant provided a flowchart of the manufacturing process. However, the FEEDAP Panel is of the view that the manufacturing process is not fully described. The applicant stated that the production is performed according to HACCP principles.

2.1.2. Manganous sulphate monohydrate

'Manganous sulphate, monohydrate' (CAS No 10034-96-5; IUPAC name: Manganous(II) sulphate, hydrate) has the chemical formula $MnSO_4 \cdot H_2O$ (molecular weight 169.01 g/mol; 32.48 % manganese). The product specification sheet indicates that the additive contains a minimum of 31 % manganese (with further specification of manganous sulphate, monohydrate ($MnSO_4 \cdot H_2O$) content minimum of 95.3 %). The additive in the application is characterised to contain 97.48 % $MnSO_4.H_2O$ (soluble in HCl); it contains also other minerals (expressed as sulphates).

The manganese content of the additive measured in five batches confirmed the specification and corresponded to about 97.2 % manganous sulphate monohydrate. The same batches were analysed for iron, copper, cobalt, zinc and molybdenum.[17]

Impurities/contaminants (lead, cadmium, mercury, arsenic, dioxins and dioxin-like PCBs) were analysed on five batches of the additive. All values were below the maximum contents set in Directive 2002/32/EC, as well as below the action thresholds for dioxins and dioxins like-PCBs. The concentrations of mercury do not give rise to safety concerns. The nickel content of the additive was analysed in ten batches; the applicant showed that the nickel monitoring is part of the HACCP implemented system.[18]

The product is a solid, white-pinky powder and odourless. It is highly soluble in water (42.5 to 45 % w/w at 20°C); bulk density is 900 kg/m^3.

[12] Technical Dossier/Section II.
[13] Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. OJ L 140, 30.5.2002, p. 10.
[14] Technical Dossier/Section II. Technical Dossier/Supplementary Information.
[15] Technical Dossier/Section II/Annex II-19- PSDMnO_conf. Technical Dossier/Supplementary Information.
[16] Technical Dossier/Supplementary Information.
[17] Technical Dossier/Section II.
[18] Technical Dossier/Section II. Technical Dossier/Supplementary Information.

Data on particle size distribution by laser diffraction was provided (four batches).[19] The dusting potential was measured according the Stauber-Heubach method in in two data sets of three batches.[20]

The applicant provided a flowchart of the manufacturing process which, in the view of the FEEDAP Panel, insufficiently describes the different production steps from starting material to the final product. The applicant stated that the production is performed according to HACCP principles.

2.2. Stability and homogeneity

Stability of inorganic compounds of trace elements needs not be demonstrated.

The applicant provided a study which demonstrated the capacity of the homogeneous distribution of the additives (manganous oxide and manganous sulphate monohydrate) in feed.

2.3. Physico-chemical incompatibilities in feed

Based on 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 when formulating diets.

2.4. Conditions of use

Manganous oxide and manganous sulphate monohydrate are intended for use in feed for all animal species and categories without time restrictions and up to 100 mg total Mn/kg for fish and 150 mg total Mn/kg for other species. These levels are in accordance with the current authorisation.

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

EFSA has verified the EURL report as it relates to the methods used for the control of manganese (six compounds, including manganous oxide and manganous sulphate monohydrate) in animal feed. The Executive Summary of the EURL report can be found in Annex A.

3. Safety

3.1. Safety for the target species

Tolerance studies are not required for compounds of trace elements already authorised (Regulation (EC) No 429/2008).[21] The assessment of manganese safety for the target species is based on a previous opinion of the Scientific Committee on Food (SCF) (EC, 2000) and on more recent FEEDAP Panel opinions (2009a, 2010).

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 dry matter (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

[19] Technical Dossier/Section II/Annex II-20- PSDMnSO4.H2O_conf. Technical Dossier/Supplementary Information.
[20] Technical Dossier/Supplementary Information.
[21] 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.

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).

Since manganous oxide and manganous sulphate monohydrate contain nickel as a contaminant, the FEEDAP Panel assessed the impact of nickel on the safety for the target species. Considering the background levels in animal feed being 0.5–4 mg/kg DM feed (Nicholson et al., 1999; Van Paemel et al., 2010), nickel would be incorporated by supplementing feeds with manganous oxide, at only low quantities, that is ~90 µg Ni/kg fish feed (when adding 100 mg Mn from manganous oxide) and ~130 µg Ni/kg feed for other animal species/categories (when adding 150 mg Mn from manganous oxide). Conversely, manganous sulphate monohydrate contains only very low amounts of nickel. Therefore, no concerns for the safety of the target animals would arise from this particular aspect for either additive.

The use of manganous oxide from the additive under assessment contributes to total cobalt in the diet (e.g. 100 mg supplemental manganese from MnO would add about 0.17 mg Co/kg complete feed). This may need consideration when formulating compound feed since the maximum content for cobalt in complete feed is rather low (2 mg/kg feed currently authorised by Regulation;[22] 1 mg/kg recommended by FEEDAP Panel (EFSA FEEDAP Panel, 2009b; EFSA FEEDAP Panel 2012a, 2012b, 2012c).

3.1.1. Interactions

Manganese and iron compete for absorption sites. Fibre, phytate, calcium, phosphorus and magnesium may also interfere with manganese absorption. Manganous oxide and manganous sulphate monohydrate are not expected to show any relevant interactions with feed components other than those well recognised for manganese.

3.1.2. Conclusions on safety for the target species

Manganous oxide and manganous sulphate monohydrate 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. Safety for the consumer

3.2.1. 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). The absorption rate depends on the amount present in the gut. 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 a minor route of excretion; it is of endogenous nature and therefore 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 none of these tissues is considered a valuable indicator of manganese availability in the gut (Jongbloed et al., 2002).

[22] 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.

3.2.2. Manganese deposition studies

Following Commission Regulation (EC) No 429/2008, residue/deposition studies are not required for an already approved compound of trace elements.

At the request of EFSA for supplementary information the applicant performed a literature search on tissue deposition of manganese from both manganous sulphate monohydrate and manganous oxide in comparison with other inorganic manganese sources. For terrestrial animals, the tabulated data submitted by applicant originated only from the technical report on selected trace elements procured by EFSA (Van Paemel et al., 2010); for fish, three additional published papers (Lorentzen et al., 1996; Watanabe et al., 1997; Tan et al., 2012) were provided. The main findings are summarised below.

Based on studies on animals fed diets containing manganese up to the highest authorised levels in Europe, the highest concentrations of manganese are found in the liver, pancreas and kidney; the lowest levels occur in bone and fat (Jongbloed et al., 2002; Van Paemel et al., 2010). The maximum tissue concentration of manganese described in the above-mentioned reviews are summarised in Table 2. Liver concentrations of manganese are 10- to 20-fold higher than in muscle tissue, with kidneys showing about half of the concentration found in liver.

Table 2: Highest manganese concentrations (mg/kg) reported for muscle, liver and kidney of various animal species

Tissue	Pigs[1]	Chickens and turkeys for fattening[2]	Ducks[2]	Ruminants (calves, cattle, heifers, lambs, sheep)[3]
Muscle	0.14	0.2	0.3	0.3
Liver	4.2	4.1	10.4	3.5
Kidney	1.6	2.4	2.5	1.4

(1) Leibholz et al., 1962; Coleman et al., 1992; Jorhem and Sundström, 1993; Lopez-Alonso et al., 2007; Gerber et al., 2009.
(2) Coleman et al., 1992; Gerber et al., 2009.
(3) Hidiroglou and Shearer, 1976; Coleman et al., 1992; Jorhem and Sundström, 1993; Blanco-Penedo et al., 2006; Gerber et al., 2009.

From three studies (Leblanc et al., 2005; Van Overmeire et al., 2006; Gerber et al., 2009), 0.3 mg Mn/kg egg could be derived as the highest concentration for eggs. For milk, rather low manganese levels (up to 0.1 mg/L) are reported in two studies (Santos et al., 2004; Leblanc et al., 2005). For a variety of fish species (Atlantic and Baltic herring, burbot, cod, eel, mackerel, perch, pike, picked dogfish, plaice, pollack and turbot) the reported manganese level was up to 0.4 mg/kg flesh (Engman and Jorhem, 1998). Two other publications (Alasalvar et al., 2002; Turkmen and Ciminli, 2007) reported higher levels (up to 7.3 mg Mn/kg DM, corresponding to approximately 1.8 mg Mn/kg flesh) for sea bass (both cultured and wild), as well as in some minor fish species.

Only a few studies are available regarding the dose-response of dietary manganese in edible tissue/product deposition. Essentially, no influence of supplemental inorganic manganese (from 10 to 120 mg/kg feed, background concentrations 8–16 mg Mn/kg feed) on tissue concentrations (muscle and liver) was found in studies with chickens for fattening (Lu et al., 2007) and growing cattle (Hansen et al., 2006; Legleiter et al., 2005), and on egg concentration (Mabe et al., 2003; Huyghebaert et al., 2006).

In contrast, an increased manganese deposition was observed in the tibia and heart of chickens for fattening fed a diet containing 60 mg supplemental manganese from manganous sulphate monohydrate (background 21 mg Mn/kg feed), with no further increase in deposition at higher supplementation levels (Li et al., 2004).

Manganese concentrations in the body of juvenile fish (yellow catfish (*Pelteobagrus fulvidraco*) and Atlantic salmon (*Salmo salar*)) responded to gradually increased supplemental manganese (Lorentzen et al., 1996; Tan et al., 2012). However, no studies on fish with marketable size were available.

Studies which allow a comparison of the relative bioavailability of the additives under assessment with other inorganic sources of manganese at concentrations at or below the currently authorised maximum content were not found. Studies in which manganese concentrations were more than 20 times higher than the maximum authorised were examined but are not considered representative (Black et al., 1984, 1985; Wong-Valle et al., 1989).

3.2.3. Conclusions on manganese deposition in edible tissues/products

No essential differences in the manganese deposition in edible tissues and products of animal origin will be expected from feed supplementation with manganous oxide or manganous sulphate monohydrate, provided that the currently authorised total manganese contents in complete feed are respected.

3.2.4. Toxicological profile of manganese

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.

3.2.4.1. Toxicological reviews

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 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). 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 GABA (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). A recent

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 two years to manganese from manganous sulphate at 60, 200 or 465 mg/kg body weight (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 1800 mg Mn/kg bw (males) or 200, 700 or 2250 mg Mn/kg bw (females). There was equivocal evidence[23] 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 that manganese causes cancer in humans (ATSDR, 2012).

As manganese is an essential element, oral exposure is not only unavoidable, but necessary to meet nutritional requirements.

3.2.5. Assessment of consumer safety

Ingested manganese appears to be well tolerated in humans and manganese intake is generally low (mean 4.9 mg/day; EVM, 2003). The general population is exposed to manganese through consumption of food and water, inhalation of air and dermal contact with air, water, soil and consumer products that contain manganese. The primary source of manganese intake is through the diet (ATSDR, 2012).

Anaemic individuals may be vulnerable to the toxic effects of manganese as a result of the increased absorption that occurs in states of iron deficiency. Groups with impaired biliary clearance, such as patients with liver disease or older people, may also be susceptible to manganese accumulation and toxicity. It has also been reported that ethanol and long-term use of anti-psychotic drugs increase the susceptibility of humans to manganese toxicity (EVM, 2002).

[23] 'Equivocal evidence' of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related (NTP, 1993).

The SCF (EC, 2000) could not conclude about a tolerable upper intake level (UL) of manganese owing to significant data gaps. Correspondingly, the ATSDR (2012) could not establish oral Minimal Risk Levels for acute-, intermediate-, or chronic-duration of oral exposure to manganese. However, a Tolerable Upper Intake Level for adults of 11 mg manganese/day has been set by the US Food and Nutrition Board of the Institute of Medicine (IOM) (FNB/IOM, 2001) based on an estimate that people eating vegetarian or Western-style diets consume up to 10.9 mg Mn/day with no reports of adverse effects (Greger, 1999). No recommended dietary allowance has been defined for manganese. The FNB/IOM (2001) has set adequate intake (AI) levels for manganese for humans. The AIs are 2.3 and 1.8 mg/day for males and females, respectively, and 1.2 mg/day for toddlers. Based on the dietary intake plus 4 mg/person per day from supplements, the EVM (2003) estimated a guidance value of 0.2 mg Mn/kg bw per day, corresponding to 12 mg/day for a 60-kg person. The guidance value for older people (60 kg bw) is 0.15 mg/kg bw.

The normal dietary intake is calculated to be approximately 2-5 mg/day (Jankovic, 2005; cited by IEH, 2007; Rubio et al., 2009; Turconi et al., 2009). This is in line with older data reviewed by the SCF (EC, 2000) and the EVM (2003). The dietary intake of adults has been estimated to range from 0.9 to 7 mg Mn/day (Schlettwein-Gsell and Mommsen-Straub, 1973; cited by EC, 2000), from 2 to 9 mg Mn/day (WHO-IPCS, 1981; cited by EC, 2000) and 1.2 to 9.4 mg Mn/day (Ellen et al., 1990; cited by EC, 2000). A Total Diet Study showed that the estimated average intake of manganese in the UK population in 1994 was 4.9 mg/day (97.5th percentile: 8.2 mg/day), including 2.3 mg/day from beverages (MAFF, 1997; cited by EC, 2000).

According to EPA (2003) *'*Nuts and nut products contain between 18.2 and 46.8 mg Mn/kg, grain and grain products between 0.4 and 40.7 mg, legumes between 2.2 and 6.7 mg, fruits between 0.2 and 10.4 mg, vegetables and vegetable products between 0.4 and 6.6 mg, meat (poultry, fish, eggs) between 0.1 and 4.0 mg, milk and milk products between 0.02 and 0.49 mg Mn/kg*'*. The manganese contents in food are in the same dimensions as those reported by Souci et al. (2008), who reported the following (values represent the range of averages for several animal species): milk, 0.025–0.068; muscle, 0.13–0.38; heart, brain and kidney, 0.24–1.50; liver, 2.80–3.30, whole chicken egg, 0.7; salt water fish. 0.12–0.87; fresh water fish, 0.14–0.95 mg/kg fresh matter.

Manganese intake can be higher in vegetarians because higher levels of manganese occur in food of plant origin. Tea drinkers are a potential high-intake group (a cup of tea can contain 0.4–1.3 mg manganese (WHO, 1996; cited by EC, 2000; the manganese content of black tea is reported to be 730 mg/kg dried tea (Souci et al., 2008)), although the bioavailability of manganese in tea is unclear and may be low owing to the presence of tannins. Many individuals who do not usually eat whole grains, nuts, certain fruits (pineapple), green leafy vegetables and tea will consume a 'low-manganese' diet — less than 2 mg per day (Davis et al., 1992; cited by EPA, 2003).

The SCF (EC, 2000) noted that the available data indicated that, in both humans and experimental animals, the margin between oral effect levels and the estimated intake from food is low, suggesting that oral exposure to manganese beyond that normally present in food and beverages could represent a risk of adverse health effects without evidence of any health benefit.

Since supplementation of feed for food-producing animals has a long history of use, it is suggested that the intake data referred to above include food originating from animals reared with manganese-supplemented feed. Manganous oxide and manganous sulphate monohydrate are commonly used inorganic sources of manganese feed supplementation. The level of manganese intake resulting from the consumption of tissues and products of animal origin are not of concern for the safety of consumers including more sensitive subgroups such as infants and elderly people. It is concluded that the use of manganous oxide and manganous sulphate monohydrate in animal nutrition is of no concern for the safety of consumers.

3.2.6. Conclusions on safety for consumers

Generally, dietary manganese does not appear to cause any adverse health effects in the population and has not proven to be a risk at the usual intake levels. Manganese intake resulting from the consumption of tissues and products of animal origin is low and not of concern for the safety of consumers, including more sensitive subgroups such as infants and elderly people. It is concluded that the use of manganous oxide and manganous sulphate monohydrate 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.

3.3. Safety for the users/workers

Manganese is a recognized 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 liver metabolism (ATSDR, 2012). Some studies suggest that manganese inhalation can result in adverse cognitive effects, such as attention deficit and amnesia (ATSDR, 2012). 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 incidences 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 (IgE) mediated.

In manganous oxide, ≥ 50 % of particles are of respirable size (≤ 10 μm) and the dusting potential is high (up to 1.4 g Mn/m^3); exposure of workers/users by inhalation when handling the product is likely to be high. Although the dusting potential of manganous sulphate monohydrate is in the same magnitude (up to 0.8 g Mn/m^3), the particle size distribution showed only a low percentage of particles of respirable size; the inhalable fraction (≤ 50 μm) amounted to a maximum of 20.5 %. However, considering the high water solubility of the compound, it could potentially be absorbed by the epithelium of the upper respiratory tract and become available to the circulatory system.

The Health and Safety Executive (HSE) of the UK and the Occupational Safety and Health Administration (OSHA) of the USA have set an occupational exposure standard (OES) for dust from manganese and its compounds (HSE, 2003; OSHA, 2007). The OES (8-hour time-weighted average) for dust is 5 mg Mn/m^3. Since the manganese concentration in the dust produced by manganous oxide and manganous sulphate monohydrate in the Stauber-Heubach apparatus is about 160–280 times higher than the OES, handling of the additives poses a risk to users upon inhalation exposure.

No specific studies were submitted on irritancy to skin and eyes, or dermal sensitisation for the additives under assessment. A variety of tests were provided regarding the possible irritating potential of manganous oxide (content of manganese 77.8 %), to skin and eyes *in vitro* (reconstituted human epidermis model[24] and reconstituted human corneal epithelium[25]) or *in vivo* (rabbit skin[26] and eye[27]). A skin sensitisation study was also performed using the *in vivo* mouse lymph node assay.[28] No skin or eye irritation or skin sensitisation could be observed in these assays. It should, however, be noted that the additive under assessment has a lower purity (content of manganese 60 %) compared with the compound tested in the submitted studies and that some of the impurities have not been identified. Therefore, the FEEDAP Panel finds it prudent to classify the additive manganous oxide as a potential skin and eye irritant and as a dermal sensitiser.

[24] Technical Dossier/Section III\Annex_III_75
[25] Technical Dossier/Section III\Annex_III_78
[26] Technical Dossier/Section III\Annex_III_77
[27] Technical Dossier/Section III\Annex_III_79
[28] Technical Dossier/Section III\Annex_III_85 and Annex_III_86.

Manganous sulphate was tested for skin and eye irritation *in vivo* and *in vitro*. The test material used in these assays was almost identical to the additive under assessment (manganese content 32.5 % *vs.* 32.1 %). Based on *in vitro* (reconstituted human epidermis model[29]) and *in vivo* (rabbit skin[30]) studies, the compound was not irritating to skin. Whilst there was no evidence for eye irritation in an *in vitro* model (reconstituted human corneal epithelium[31]), it caused severe and irreversible eye irritation in an *in vivo* test (rabbit eye[32]). Manganous sulphate was not tested for possible skin sensitisation, but manganous chloride was tested (Ikarashi et al., 1992; Basketter et al., 1999) and found to be not a skin sensitiser; because both compounds are water soluble and share the Mn^{2+} ion and neither the sulphate nor chloride ions are sensitisers to skin, applying the read-across methodology, it can be expected that manganous sulphate is also not a dermal sensitiser.

3.3.1. Conclusions on safety for the users/workers

The handling of manganous oxide and manganous sulphate monohydrate poses a risk to users upon inhalation exposure. The additive manganous oxide should be considered as a potential skin and eye irritant and as a dermal sensitiser. Manganous sulphate monohydrate is not irritating to skin but it is an eye irritant; it is likely not a dermal sensitiser.

3.4. 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).[33] 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 < 10–6480 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).

3.4.1. 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.

Following the technical guidance and further assuming that manganous sulphate monohydrate and manganous oxide will be completely dissociated in the gut and solubilised to Mn^{2+}, the predicted concentrations of manganese in surface water (PEC_{sw}) would range from 332 to 630 µg/L, with the highest concentrations resulting from the use of manganous oxide and manganous sulphate monohydrate in feed for lambs. This worst-case scenario suggests that supplementation of animal feeds with manganous oxide and manganous sulphate monohydrate would markedly increase the

[29] Technical Dossier/Section III\Annex_III_81.
[30] Technical Dossier/Section III\Annex_III_82.
[31] Technical Dossier/Section III\Annex_III_83.
[32] Technical Dossier/Section III\Annex_III_84.
[33] Available online : http://weppi.gtk.fi/publ/foregsatlas/

background concentrations of manganese in surface waters. However, this outcome is considered unrealistic because the calculated worst-case PEC_{soil} is much lower than background concentrations, and it is likely that manganese added to the soil through the spreading of manure would oxidise to insoluble (III) and (IV) forms and otherwise would behave similarly to that naturally present. Thus, the use of manganous oxide and manganous sulphate monohydrate in feeds for terrestrial animals up to a concentration of 150 mg/kg complete feed is considered safe to the aquatic compartment.

3.4.2. 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 manganous oxide and manganous sulphate monohydrate 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 212 µg/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 4000 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 1.0–2.0 µg/L, depending on the species. This concentration exceeds the trigger value by one order of magnitude, but is also one order of magnitude lower than the median concentration of manganese in European freshwaters (FOREGS).

3.4.3. Conclusions on environmental safety

The use of manganous oxide and manganous sulphate monohydrate 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.

4. Efficacy

According to Regulation (EC) No 429/2008, no efficacy studies are required for compounds of trace elements already authorised as feed additives.

The use of manganous oxide and manganous sulphate monohydrate 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.

5. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[34] and Good Manufacturing Practice.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Manganous oxide and manganous sulphate monohydrate are considered safe for all animal species/categories, provided that the maximum total contents of manganese authorised in feed (100 mg/kg complete feedingstuffs for fish and 150 mg/kg for other species) are respected.

Generally, dietary manganese does not appear to cause any adverse health effects in the population and has not proven to be a risk at the usual intake levels. Manganese intake resulting from the

[34] 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.

consumption of tissues and products of animal origin is low and not of concern for the safety of consumers, including more sensitive subgroups such as infants and elderly people. It is concluded that the use of manganous oxide and manganous sulphate monohydrate 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.

The handling of manganous oxide and manganous sulphate monohydrate poses a risk to users upon inhalation exposure. The additive manganous oxide should be considered as a potential skin and eye irritant and as a dermal sensitiser. Manganous sulphate monohydrate is not irritating to skin but it is an eye irritant; it is likely not a dermal sensitiser.

The use of manganous oxide and manganous sulphate monohydrate in animal nutrition for all animal species is not of concern for the environment, provided that the current maximum total contents of manganese authorised in feed are respected.

Manganous oxide and manganous sulphate monohydrate are efficacious sources of manganese in meeting animals' requirements.

RECOMMENDATIONS

The *Description and conditions of use of the additive as proposed by the applicant* should be amended as follows:

- The description of the additives should include (i) the details for manganous sulphate monohydrate, (ii) the minimum assay of manganous oxide (e.g. 79 % HCl soluble MnO) and manganous sulphate monohydrate (e.g. 97 % HCl soluble $MnSO_4 \cdot H_2O$) in each additive and (iii) the minimum content of manganese in the additives, e.g. 60 % Mn in the additive manganous oxide and 30 % Mn in the additive manganese sulphate monohydrate.
- Other provisions should contain:
  - The incorporation of the additives into feed should be made via premixtures only, to ensure user safety.
  - The contribution of cobalt from the manganous oxide under application to the total cobalt content in feed should be considered when formulating compound feeds.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier Manganese (Nutritional additive – Compound of trace element – E 5): MnO and $MnSO_4.H_2O$ for all animal species. October 2010. Submitted by Eramet & Comilog Chemicals S.A.

2. Dossier Manganese (Nutritional additive – Compound of trace element – E 5): MnO and $MnSO_4.H_2O$ for all animal species. Supplementary information. May 2013. Submitted by Eramet & Comilog Chemicals S.A.

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

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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)

Health Canada Agency – Human health risk assessment for inhaled manganese (http://www.hc-sc.gc.ca/ewh-semt/pubs/air/manganese-eng.php)

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 feed additive

The following manganese compounds are authorised for use in food (Regulation (EC) No 1170/2009):[35] 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):[36] 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):[37] 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[38] 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[39] these compounds are for oral use only.

The following manganese compound is listed in Annex of Commission Implementing Regulation (EU) No 540/2011[40] 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[41] 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).

[35] 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.

[36] 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.

[37] 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.

[38] 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

[39] 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.

[40] 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

[41] 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):[42] ammonium manganese (3+) diphosphate and trimanganese bis (orthophosphate).

According to the Annex of Regulation (EC) No 432/2012[43] 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[44]: 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.

[42] 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.

[43] 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.

[44] 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.

Annex A. Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Manganese (E5)[1]

In the current application authorisation is sought under articles 4(1) and 10(2) for *manganese chelate of glycine hydrate*[2], *manganese chelate of amino acids hydrate*[2,3], *manganous oxide*[2,4,5], *manganous carbonate*[2], *manganous chloride tetrahydrate*[2] and *manganous sulfate monohydrate*[2,5] 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 sulfate 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 sulfate 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 sulfate 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 sulfate 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

[1] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/default/files/FinRep-SANCO-Manganese.pdf
[2] FAD-2010-0088.
[3] FAD-2010-0069.
[4] FAD-2010-0166.
[5] FAD-2010-0235.

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.

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 sulfate monohydrate* (FAD-2010-0088).