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SCIENTIFIC OPINION

Scientific opinion on the safety and efficacy of iron compounds (E1) as feed additives for all species: iron chelate of amino acids, hydrate, based on a dossier submitted by Zinpro Animal Nutrition Inc.[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 10 January 2014, replaces the earlier version published on 10 July 2013.[4]

ABSTRACT

The use of iron chelate of amino acids, hydrate, as source of iron is considered safe for all animal species/categories when used up to the currently authorised maximum content of total iron in complete feed, with the exception of bovines and poultry for which the maximum tolerated level is 450 mg/kg complete feed, and pets, for which the maximum tolerated level is 600 mg/kg complete feed. The FEEDAP Panel is not in the position to derive a maximum safe iron concentration in feed for horses or fish. Consumption surveys include iron-containing foodstuffs of animal origin. Since the supplementation of animal feed with iron-containing compounds has not essentially changed during the last decades, it is reasonable to assume that the iron levels in food of animal origin used in exposure scenarios originated from animals fed iron-supplemented diets. Since iron chelate of amino acids, hydrate, will be used as a substitute for other iron compounds, its use in animal nutrition would not modify consumer exposure to iron. The additive should be considered as a skin, eye and respiratory irritant and, owing to its residual peptide component, as a skin/respiratory sensitiser. Considering the high background concentration of iron in soil and water, the supplementation of feed with iron chelate of amino acids, hydrate, is not expected to pose an environmental risk. Iron chelate of amino acids, hydrate, is an effective source of iron for all animal species and categories. The FEEDAP Panel recommends that the maximum iron contents in complete feed be reduced as follows: bovines and poultry, 450 mg Fe/kg; and pets, 600 mg Fe/kg.

KEY WORDS

nutritional additive, compounds of trace elements, iron, 'iron chelate of amino acids, hydrate', safety, environment, efficacy

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[1] On request from the European Commission, Question No EFSA-Q-2012-00490, adopted on 19 June 2013.

[2] Panel members: Gabriele Aquilina, Alex Bach, 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] Revision 1: erratum. The recommendation concerning the molecular weight of the compound has been redefined.

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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 iron chelate of amino acids, hydrate, when used as feed additive for all animal species.

Iron serves as a constituent in proteins (e.g. haemoproteins: haemoglobin, myoglobin; non-haemo proteins: ferritin, transferrin) and as a cofactor for many important iron-dependent enzymes (e.g. cytochromes A, B, C; peroxidases, catalases).

The use of iron chelate of amino acids, hydrate, as source of iron, is considered safe for all animal species/categories when used up to the currently authorised maximum content of total iron in complete feed (ovine: 500 mg Fe/kg complete feedingstuffs; piglets up to one week before weaning: 250 mg Fe/day; other species: 750 mg Fe/kg complete feedingstuffs), with the exception of bovines and poultry for which the maximum tolerated level is 450 mg/kg complete feed, and pets, for which the maximum tolerated level is 600 mg/kg complete feed. The FEEDAP Panel is not in the position to derive a maximum safe iron concentration in feed for horses or fish.

Consumption surveys include iron-containing foodstuffs of animal origin. Since the supplementation of animal feed with iron-containing compounds has not essentially changed during the last decades, it is reasonable to assume that the iron levels in food of animal origin used in exposure scenarios originated from animals fed iron-supplemented diets. Since iron chelate of amino acids, hydrate, will be used as a substitute for other iron compounds, its use in animal nutrition would not modify consumer exposure to iron.

The additive should be considered as a skin, eye and respiratory irritant and, owing to its residual peptide component, as a skin/respiratory sensitiser.

Considering the high background concentration of iron in soil and water, the supplementation of feed with iron chelate of amino acids, hydrate, is not expected to pose an environmental risk.

Iron chelate of amino acids, hydrate, is an effective source of iron for all animal species and categories.

The FEEDAP Panel recommends that the maximum iron contents in complete feed be reduced as follows: bovines and poultry, 450 mg Fe/kg; and pets, 600 mg Fe/kg.

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BACKGROUND

Regulation (EC) No 1831/2003[1] 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 Zinpro Animal Nutrition[2] for re-evaluation of authorisation of the iron-containing additive iron chelate of amino acids, hydrate, (Availa®Fe) 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.[3] 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 24 of May of 2012.

The additive "Ferrous chelate of amino acids, hydrate" had been authorised in the EU under the element Iron-Fe for all animal species "Without a time limit" (Commission Regulation (EC) No 1334/2003)[4] and amendments, and Commission Regulation (EC) No 479/2006.[5] 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".[6]

EFSA issued an opinion on the safety of the chelated forms of iron, copper, manganese and zinc with synthetic feed grade glycine (EFSA, 2005).

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and the efficacy of the Iron chelate of amino acids, hydrate, when used under the conditions described in Table 1.

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] Zinpro Animal Nutrition Inc. Akkerdistel 2E, 5831 PJ-Boxmeer, The Netherlands.
[3] EFSA Dossier reference: FAD-2010-0068.
[4] Commission Regulation (EC) No 1334/2003 of 25 July 2003 amending the conditions for authorisation of a number of additives in feedingstuffs belonging to the group of trace elements. OJ L 187, 26.7.2003, p. 11.
[5] Commission Regulation (EC) No 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.
[6] 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	Availa®Fe
Registration number/EC No/No (if appropriate)	
Category(-ies) of additive	Nutritional additive
Functional group(s) of additive	3(b)

Description			
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)
Iron Amino Acid Chelate, Hydrate	Fe $(x)_{1\text{-}3}\cdot nH_2O$ (x = anion of any amino acid derived from hydrolysed soya protein)	9% Iron	CEN/TS 15621:2007; *Animal feeding stuffs - Determination of calcium, sodium, phosphorus, magnesium, potassium, sulphur, iron, zinc, copper, manganese, cobalt and molybdenum after pressure digestion by ICP-AES*

Trade name (if appropriate)	Availa®Fe
Name of the holder of authorisation (if appropriate)	Zinpro Animal Nutrition Inc., The Netherlands

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	-	Ovine	500 (total)	Not relevant
		Pet animals	1250 (total)	
		Piglets up to one week before weaning	250 mg/day (total)	
		Other species	750 (total)	

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use (if appropriate)	None
Specific conditions or restrictions for handling (if appropriate)	For user safety: gloves, respiratory and eye protection recommended. (see MSDS)
Post-market monitoring (if appropriate)	Tracking & tracing will be in full place, all remarks during the use of Availa® Fe will be noticed and recorded.
Specific conditions for use in complementary feedingstuffs (if appropriate)	Availa® Fe can be mixed in feeds, to supply Fe in final feed within EU legal limits for each species.

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

ASSESSMENT

This opinion is based in part on data provided by a single company involved in the production/distribution of iron chelate of amino acids, hydrate. It should be recognised that these data cover only a fraction of iron chelates of amino acids, hydrate, on the market. The FEEDAP Panel has sought to use the data provided together with data from other sources to deliver an opinion.

The FEEDAP Panel assessment is based on data provided by the applicant for the specific product under application, and its conclusions cannot be fully extended to all iron chelate of amino acids, hydrate, on the market.

1. Introduction

Iron is the most abundant trace element in mammals. It serves as a constituent in proteins (e.g. haemoproteins: haemoglobin, myoglobin; non-haemo proteins: ferritin, transferrin) and as a cofactor for many important iron-dependent enzymes (e.g. cytochromes A, B, C; peroxidases, catalases). Haemoglobin makes up 80 % of the entire iron body pool. The intestinal absorption of iron and its retention is highly regulated via homeostasis (for reviews see Wessling-Resnick, 2000; Miret et al., 2003; Fuqua et al., 2012). Iron is present in biological systems in one of two oxidation states, and redox interconversions of the ferrous (Fe(II)) and ferric (Fe(III)) forms are central to the biological properties of this trace element. Aerobic metabolism depends on iron. As a constituent of haemoglobin, it is involved in oxygen and carbon dioxide transport. It plays a central role as cofactor for most of the enzymes of the Krebs cycle and functions as electron carrier (McDowell, 2003; Suttle, 2010).

The application is sought for the use of iron chelate of amino acids, hydrate, in feed for all animal species/categories. This compound, a source of organic iron, is already authorised in the EU as nutritional additive.

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

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

2. Identity and characterisation

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

Iron chelate of amino acids, hydrate, has the generic chemical formula $Fe(x)_{1-3} \cdot nH_2O$, where x= anion of any amino acid. No full information was made available on the oxidation state of the iron in the chelate. Therefore, in this opinion, it is further referred to as iron chelate of amino acids, hydrate, rather than as a denomination (ferrous) which indicates the valency of iron. The compound under application is derived from hydrolysed soybean protein, molecular weight not exceeding 1500 Da.

2.1. Manufacturing process

The manufacturing process comprises an acid hydrolysis of soybean protein under heat, followed by chelation with a source of bivalent iron. The process is optimised to maximise the release of free

amino acids.[7] The final additive is obtained by adding cellulose (44–45 %) and calcium carbonate (19–20 %) to the slurry and subsequent drying.

The applicant stated that the soybean used in the production of the additive is of conventional origin, non-genetically modified, and either contractually sourced, or based on an approved geographic region.[8]

2.2. Characterisation of the iron compound

The iron content of five batches of the slurry[9] ranged from 8.45 to 8.61 %.[10] The applicant estimated the molecular weight of the compound to be 166–271 Da.[11] Analytical data on one sample showed that 95.5 % of the chelated material had a molecular weight < 1500 Da and 4.5 % a molecular weight > 1500 Da.[12] The lysinoalanine content in five batches of the hydrolysate was below 5 mg/100 g crude protein.[13] The content of heavy metals (lead, cadmium and mercury), fluorine and arsenic analysed in the slurry is in accordance with Directive 2002/32/EC,[14] as are the dioxins and the sum of dioxins plus dioxin-like polychlorinated biphenyls (PCBs) (all values derived from three batches).[15]

Analytical data on five batches of the hydrolysate material before the addition of the iron source showed that at least about 95 % of the total amino acid content is present in form of free amino acids.[16]

2.3. Characterisation of the additive

The final product is specified to contain a minimum of 9 % iron, as confirmed by analysis of five batches (range 9.63–9.75 % iron).[17] Proximate analysis of one batch revealed a content of 8.7 % moisture, and on a dry matter (DM) basis: 26.4 % crude protein (nitrogen × 6.25), 3.7 % lipids, 1.3 % crude fibre and 49.1 % ash; the mineral fraction consists of 26 % sulphur, 14 % sodium, 0.2 % calcium, 0.1 % potassium and 0.08 % phosphorus.[18,19]

Impurities were analysed in five batches. Heavy metals (lead, cadmium and mercury), fluorine and arsenic complied with the limits set in Directive 2002/32/EC, as did dioxins and the sum of dioxins plus dioxin-like PCBs.[20] The nickel content was between 17 and 27 mg/kg additive.[21] In three batches *Escherichia coli* O157:H7 and *Salmonella* (25 g sample of the product) were absent.[22]

2.4. Physical state of the additive

The product is a coarse granular powder. It is reported to be insoluble in water. Bulk density is 670 kg/m^3.

[7] Technical Dossier/Supplementary Information.
[8] Technical Dossier/Supplementary Information.
[9] Slurry means the intermediate product of iron chelation with the protein hydrolysate.
[10] Technical Dossier/Supplementary Information.
[11] Technical Dossier/Section II.
[12] Technical Dossier/Supplementary Information.
[13] Technical Dossier/Supplementary Information.
[14] Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. OJ L 140, 30.5.2002, p. 10.
[15] Technical Dossier/Supplementary Information.
[16] Technical Dossier/Supplementary Information.
[17] Technical Dossier/Section II.
[18] Technical Dossier/Section II/Annex II_ 2_a.
[19] The analytical values submitted by the applicant showing the typical composition of the final additive, do not match the additive composition (i.e. the calcium content is inexplicably low considering the amount of calcium carbonate added). Only two explanations for such a discrepancy could be possible: the composition of the additive is different to that given in the application or the analytical data are from another additive. Since an application for a nutritional additive is not holder specific, the composition of the additive is not subject to EFSA assessment, since no safety concerns are involved.
[20] Technical Dossier/Section II/Annex II_ 2_b (three annexes); Technical Dossier/Supplementary Information.
[21] Technical Dossier/Supplementary Information.
[22] Technical Dossier/Section II.

Sieve analysis of one batch identified that 0.4–1.0 % (w/w) of particles had a diameter below 180 µm.[23] At the request of the FEEDAP Panel, sieve analysis was repeated in three other batches, and revealed the fraction below 75 µm to be 0.0–0.5 % (w/w).[24] No data on dusting potential were provided.

2.5. Stability

No data on stability were provided for the iron chelate amino acids, hydrate. Information on the maintenance of the specific iron bonds in the chelates would be valuable. The FEEDAP Panel recognises the analytical difficulties in demonstrating stability of this specific bond and notes that the active substance is also unlikely to disappear in these products.

2.6. Homogeneity

The potential homogeneous distribution of the additive was predicted using a statistical approach (Jansen, 1992) in the case of a complete feed for chickens for fattening. The coefficient of variation was 2.6 %.[25] However, the FEEDAP Panel notes that this method has been developed to test the working accuracy of mixing equipment and its applicability to test homogeneity has not been demonstrated.

2.7. Physico-chemical incompatibilities in feed

Based on current knowledge, no incompatibilities resulting from the use of iron in compound feed are expected, other than those widely known and considered by feed manufacturers when formulating diets.

2.8. Conditions of use

The iron compound under application, iron chelate of amino acids, hydrate, is intended to supply iron in final feed for all animal species/categories up to a maximum total content of 250 mg/day for piglets up to one week, 750 mg/kg complete feedingstuffs for other pigs, 500 mg/kg complete feedingstuffs for ovines, 1250 mg/kg complete feedingstuffs for pets and 750 mg/kg complete feedingstuffs for all other animal species.

2.9. 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 the iron (eight compounds, including ferrous/iron chelate of amino acids, hydrate) in animal feed. The Executive Summary of the EURL report can be found in Appendix A.

3. Safety

3.1. Safety for the target species

3.1.1. Safety of iron for animal species

Before assessing the safety of the compound under application, the FEEDAP Panel made a comparison with the currently authorised maximum iron (total) contents set by Regulation (EC) No 1334/2003, which should be considered safe for the target animals, and the maximum tolerated levels (MTLs) published by the National Research Council of the USA in 2005 (NRC, 2005). The discrepancies observed are in 'other animal species'—which covers, in the case of iron, all species except 'Ovine', 'Pet animals' and 'Piglets up to one week before weaning'—where the EU Regulation allows 750 mg/kg complete feedingstuffs, but the NRC considers only 500 mg/kg feed DM as safe for

[23] Technical Dossier/Section II.
[24] Technical Dossier/Supplementary Information.
[25] Technical Dossier/Section II.

cattle, poultry and horses (and rodents). The MTL for pigs, at 3000 mg/kg DM, is considered to exceed that set in the EU Regulation.

The FEEDAP Panel considered in depth the literature cited in the NRC publication to identify the basis for the lower MTLs for cattle, poultry and horses (and rodents).

For cattle (including steers) and dairy cows (including calves) three studies were reviewed (Standish et al., 1969; Koong et al., 1970; Jenkins and Hidiroglou, 1987). The FEEDAP Panel arrived at the same conclusions as NRC that concentrations above 500 mg Fe/kg DM would result in adverse effects, primarily expressed as reduced feed intake and partially combined with sign of copper deficiency. The Panel identified studies in which levels as low as 250 mg Fe/kg feed reduced copper content in liver (Bremner et al., 1987). Iron is known to be an important copper-antagonist (Standish et al., 1969, 1971; Standish and Ammerman, 1971; Campbell et al., 1974; Prince et al., 1979), but also to zinc and manganese (Grün et al. 1978). However, considering that cattle obtain iron from a variety of sources, including grazing, it would be impractical to impose a maximum iron content in feed of less than 500 mg Fe/kg DM. Thus, the FEEDAP Panel concludes that 450 mg Fe/kg complete feedingstuffs (88 % DM) is safe for bovines.

One-day old chickens for fattening were fed diets (background content 188 mg Fe/kg DM) supplemented with 400, 600 or 800 mg iron from ferrous sulphate heptahydrate, reagent grade, per kg feed for up to three weeks (Cao et al., 1996). Increasing iron concentration resulted in decreased feed intake and delayed growth; the effects were more pronounced at higher iron supplementation. The results would identify 565 mg Fe/kg complete feed as a low observed adverse effect level (LOAEL). Considering the differences in the iron availability of background and supplemented iron in practice, the FEEDAP Panel could accept the NRC conclusion that 450 mg Fe/kg complete feed (88 % DM) is safe for poultry.

No studies on adult horses were found in the NRC publication. However, studies in newborn Shetland foals indicated a high sensitivity of newborn animals to high iron doses (16.5 mg Fe/kg bw, given orally as ferrous fumarate) (Mullaney and Brown, 1988, cited by NRC, 2005). About the 3-fold dose has been tolerated for four weeks by adult ponies (Pearson and Andreasen, 2001, cited by NRC, 2005). The FEEDAP Panel is not in the position to derive a maximum safe iron concentration in feed from the data available.

The maximum content of iron set for ovines in the EU (500 mg/kg complete feed) is not essentially different from the MTL (500 mg/kg feed DM) given by the NRC in 2005. This limit is confirmed by earlier studies (Flachowsky et al., 1976; Grün et al., 1978) not considered by the NRC.

A study on Atlantic salmon concluded that an iron inclusion level of 400 mg Fe (as iron sulphate)/kg feed was tolerated (Andersen et al., 1996). This conclusion is supported by a survey of iron levels in commercial fish feeds used in Norway between 2004 and 2009, which found the maximum reported level to be 493 mg Fe/kg feed (Måge et al., 2010). The scarce information available does not indicate a need for a revision of the maximum currently authorised iron level in feed for fish. However, in agreement with the NRC, the FEEDAP Panel notes that there are insufficient data to conclude on the iron tolerance of fish.

Although iron is commonly added to pet food, information on the toxicity is limited. No clinical signs of toxicosis are expected in dogs ingesting less than 20 mg/kg bw ionisable iron. Dogs ingesting between 20 and 60 mg/kg bw elemental iron can develop mild clinical signs. The most affected organ is the liver. When the amount of elemental iron ingested is greater than 60 mg/kg bw, serious clinical signs can develop. Oral doses between 100 and 200 mg/kg bw are potentially lethal (Osweiler et al., 1985; Greentree and Hall, 1995, cited by Albretsen, 2006). After application *per os* to dogs a dose of 150 mg/kg bw ferrous sulphate, Reissmann and Coleman (1955) observed a significant decrease in plasma pH (from 7.34 to 7.16). Doses of 375 mg iron from ferrous gluconate/kg bw had adverse effects on the gastrointestinal tract; similarly, 300 mg iron from ferrous sulphate/kg bw also affected

the gastrointestinal tract. Higher doses of ferrous sulphate (600 mg Fe/kg bw) caused death, but 1500 mg iron from ferrous carbonate/kg bw had no effect in dogs (D'Arcy and Howard, 1962). In mongrels, doses of 200–300 mg/kg bw intrajejunally were lethal in 5–6 h (Bronson et al., 1960). The median lethal dose (LD_{50}) of ferrous sulphate heptahydrate in cats was estimated to be 800 mg/kg bw (Hoppe et al., 1955). Applying the default values for feed intake and body weight as published in the guidance for sensory additives (EFSA, 2012), dogs would be expected to tolerate 1200 mg Fe/kg complete feed. Considering potential differences among breeds concerning sensitivity to trace elements, introducing a safety factor of 2 appears appropriate. The FEEDAP Panel concludes that 600 mg Fe/kg complete feed is safe for dogs and extends this conclusion to cats, based on acute toxicity data.

The newly proposed maximum contents for total dietary iron refer to fractions of different bioavailability, the background fraction for which a relative bioavailability compared with iron sulphate of 25 % can be assumed, and the supplemented fraction with a relative bioavailability of 100 %. The availability of commonly used iron compounds including those authorised in the EU, except ferric oxide and ferrous carbonate, does not essentially differ from the availability of iron sulphate (Henry and Miller, 1995; Jongbloed et al., 2002). Ferric oxide and ferrous carbonate are less available. Consequently, the availability of the sources of supplemented iron will have no impact on the safety of the newly proposed iron content in feed.

3.1.2. Safety of iron chelate of amino acids, hydrate, for the target species

The source of iron under application has comparable bioavailability to the iron compounds used in the toxicological studies. The newly proposed maximum safe content in feed would therefore apply.

Since the additive contains nickel as a contaminant, the FEEDAP Panel assessed the impact of nickel on safety for the target species. Considering the background levels in animal feed as 500–4000 µg/kg DM feed (Nicholson et al., 1999; Van Paemel et al., 2010), nickel would be incorporated by supplementing feeds with iron chelate of amino acids, hydrate, at an extremely low quantity, e.g. 21–34 µg Ni/kg pet feed, if adding 1250 mg Fe from iron chelate of amino acids, hydrate. Therefore, no concern for the safety of target animals would arise from this particular aspect.

3.1.3. Microbiological studies

The antimicrobial resistance conferred by the presence of iron in the additive was tested against five tetracycline-sensitive microbial strains (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 6633, as recommended by the FEEDAP Guidance on Microbial Studies; EFSA, 2008a). The minimum inhibitory concentration for all five strains was above 300 mg Fe/L, the highest tested iron concentration.[26]

3.1.4. Conclusions on the safety for target species

Iron chelate of amino acids, hydrate, is safe for all animal species/categories when used up to the currently authorised maximum content of total iron in complete feed (ovine: 500 mg Fe/kg complete feedingstuffs; piglets up to one week before weaning: 250 mg Fe/day; other species: 750 mg Fe/kg complete feedingstuffs), with the exception of bovines and poultry for which the maximum tolerated level is 450 mg/kg complete feed, and pets, for which the maximum tolerated level is 600 mg/kg complete feed. The FEEDAP Panel is not in the position to derive a maximum safe iron concentration in feed for horses or fish.

[26] Technical Dossier/Section III/Annex III_1.

3.2. Safety for the consumer

3.2.1. Absorption, distribution, metabolism and excretion

Iron chelate of amino acids, hydrate, most probably contains prevalently Fe(II) and dissociates easily. Divalent iron can be directly transported through the apical membrane by Divalent Metal Transporter-1 (DMT1), unlike Fe(III) which needs the presence of the ferrorreductase, duodenal cytochrome B (Fe(III) to Fe(II)) (see reviews by Gunshin et al., 1997 and Lawen and Lane, 2013).

The transport of iron from enterocytes to blood depends on the iron pool in the liver. A high level of iron results in the synthesis of large amounts of hepcidin in liver. Hepcidin reduces the movement of iron across the enterocytes by causing ubiquitination and subsequent proteasomal degradation of both DMT1 (Brasse-Lagnel et al., 2011) and ferroportin (De Domenico et al., 2007), which is the basolaterally located iron exporter that transports iron from the enterocyte to the circulation (Donovan et al., 2000). Simultaneously, a high iron pool decreases synthesis of hephaestin which converts Fe(II) to Fe(III) in the baso-lateral membrane, and only Fe(III) can bind to transferrin and be transported in blood (see reviews by Gunshin et al., 1997 and Lawen and Lane, 2013).

Owing to the strong regulation of its intestinal absorption, a high oral intake of iron results in a less than proportional increase in iron. Under physiological conditions intestinal iron absorption is regulated according to demand; the low renal excretion helps to maintain iron stores (Wessling-Resnick, 2000; Miret et al., 2003; EFSA, 2004; Fuqua et al., 2012). The haemoglobin concentration in blood closely reflects the amount of iron utilised in the organism and is a biomarker of a potentially deficient iron status. Ferritin binds intracellular iron which is not utilised in the functional iron pool; serum transferrin is regarded a reliable indicator of iron stores, but is also influenced by factors such as chronic diseases (EFSA, 2004).

3.2.2. Iron deposition in tissues and products of animal origin

Approximately 70 % of body iron is stored as haemoglobin. Preferential sites of deposition are liver and kidney (EFSA, 2004). Concentrations of iron in liver, kidney, muscle, eggs and milk in animal tissues and products from various food composition tables are reported in Appendix D.

Following a request from EFSA, the applicant performed a literature search for peer-reviewed articles published between 2003 and November 2012 on potential differences in the deposition in edible tissues and products of iron from inorganic and organic sources.[27] The databases used for this research were Medline, Food Science and Technology, Agricola, Biological Abstracts, Embase, Medline preprints and Life Science Collections, and included the applicant's own database. The keywords applied were reported. Only studies allowing a comparison between edible tissue/animal products deposition of iron from organic and inorganic sources were considered.

No suitable studies allowed the comparison of iron content in milk from dairy cows fed organic or inorganic iron. The content of iron in milk is low (on average 0.8 mg/kg) and remains quite uninfluenced by dietary iron concentrations (Knowles et al., 2006). Two studies in sows (Wei et al., 2005; Bertechini et al., 2012) showed a 30 % increase of iron in milk when organic iron supplements were used, but it is unclear whether such findings could be relevant to dairy ruminants.

The content of iron in whole egg is approximately 30 mg/kg (Park et al., 2004, 2005). The same authors reported a significant increase (14-18 %) of iron concentration in whole egg when comparing organic iron and inorganic iron supplements; such increase resulted in an average iron concentration of 29.6 mg/kg.

Studies in chickens (Seo et al., 2008; Petrovič et al. 2010) and pigs (Novotny et al., 2003; Pietruszka et al., 2009) did not show a consistent increase of iron concentrations in meat as a result of organic iron

[27] Technical Dossier/Supplementary Information.

supplementation. The greatest increases, reported by Seo et al. (2008), were as +34.5, +18.4 and +34.6 %, in breast, thigh and wing muscle, respectively. The same lack of consistent results was observed for iron concentrations in the liver and kidney of pigs (Feng et al., 2009; Bertechini et al., 2012) and chickens for fattening (Seo et al., 2008; Shinde et al., 2011): the greatest mean increases were +22 % and +31.9 % in broiler muscle and liver, respectively. Apparently no studies were available as regards the effect of organic iron supplementation on the iron content of muscle and offals from ruminants.

The literature reviewed indicates that at current level of feed supplementation, the use of organic iron compounds could result in an increase in iron deposition in the order of 15 % in eggs and 20–35 % in pig and chicken liver and muscle. However, the FEEDAP Panel considers that the data provided by the applicant are insufficient to fully characterise the impact of organic iron compounds on iron deposition in edible tissues and products, particularly in relation to the dietary iron concentrations. Different compounds and feed concentrations may, at least partly, explain the observed discrepancies.

Two studies in which the deposition of iron in edible tissues and eggs resulting from supplementation with an iron chelate of amino acids or iron sulphate were submitted by the applicant, one in piglets (Yu et al., 2000; product tested with 9 % iron) and another in laying hens (Park et al., 2004; product tested with 6 % iron). The deposition data are summarised below.

Groups of six piglets received a maize-soybean-whey diet (43 mg Fe/kg) supplemented with iron sulphate (100 mg Fe/kg) and further supplemented with graded levels of iron from iron chelate (30, 60, 90 or 120 mg Fe/kg feed) or iron sulphate (120 mg Fe/kg) for five weeks. Both supplements resulted in an increase in the iron concentration in liver, spleen and muscle tissue compared with the control group. Liver iron in the iron sulphate group was not significantly different from that of the group supplemented with iron chelate of amino acids group (120 mg supplemental Fe/kg). The iron concentration in spleen and muscle was higher (31 and 15 %, respectively) in the group receiving iron chelate of amino acids, but the difference was not significant ($P > 0.1$).

Groups of eight laying hens were fed a maize-soybean diet (52.5 mg/kg) supplemented with three levels of iron (100, 200 or 300 mg Fe/kg) from iron chelate or iron sulphate for 40 days. The iron content of the eggs at the end of the study was not significantly different between the two iron sources at supplementation levels of 200 and 300 mg Fe/kg. At the lowest supplementation level (100 mg Fe/kg), eggs from hens in the iron chelate group contained significantly more iron (7 %).

The two studies submitted allowing a comparison of iron chelate of amino acids and iron sulphate, as the standard, do not indicate that iron from the chelate compound would result in significantly higher tissue and product concentrations; however, a trend towards a more efficient tissue deposition from iron chelate of amino acids cannot be fully excluded. The small number of animals and the restricted number of comparisons at the same supplemental level prevent a more precise conclusion.

3.2.3. Assessment of consumer safety

The US Institute of Medicine (IOM) in 2001 proposed a tolerable upper intake level (UL) of 45 mg/day based on gastrointestinal symptoms, the most evident acute effect; this UL represented more than 5-fold the recommended dietary allowance for men and postmenopausal women (8 mg/day) and approximately 3-fold that for premenopausal women (18 mg/day) (IOM, 2001). The 27th JECFA set a provisional maximum tolerable daily intake for iron from all sources, except iron oxides used as colouring agents, of 0.8 mg/kg bw (WHO, 1983).

In 2004, the EFSA NDA Panel assessed the possible UL of iron, considering evidence of adverse effects from animal studies and human trials. In the summary of the opinion, the Panel presented the following conclusions: "*Adverse gastrointestinal effects have been reported after short-term oral dosage at 50-60 mg daily of supplemental non-haem iron [...]. Iron overload with clinical symptoms, including liver cirrhosis, has been reported in individuals receiving long-term, high-dose medical*

treatment with iron (160-1200 mg iron/day). Iron overload with clinical symptoms has also been found in subjects homozygous for hereditary haemochromatosis (a genetic disorder of iron storage), even at normal dietary iron intakes [...]. Although a proportion of the population has serum ferritin levels indicative of elevated iron stores [...] the point at which an elevated serum ferritin level becomes associated with an increased risk of adverse effects (such as liver fibrosis) is not known. The risk of adverse effects from iron overload in the general population, including those heterozygous for hereditary haemochromatosis, is considered to be low. Epidemiological studies have reported associations between high iron intake and/or stores with increased risk of chronic diseases [...]. However, these data are conflicting and do not provide convincing evidence of a causal relationship [...]. The Panel considered that the available data are insufficient to establish a tolerable upper intake level for iron. Based on estimates of current iron intakes in European countries, the risk of adverse effects from high iron intake from food sources, including fortified foods in some countries, but excluding supplements, is considered to be low for the population as a whole, except for those homozygous for hereditary haemochromatosis (up to 0.5 % of the population). However, intake of iron from food supplements in men and postmenopausal women may increase the proportion of the population likely to develop biochemical indicators of high iron stores. Some groups at special risk for poor iron status, such as menstruating women or children, could benefit from additional iron intake and/or improved availability of dietary iron."

In addition to the above conclusions of the NDA Panel, the FEEDAP Panel has discussed the available evidence for iron genotoxicity, concluding that positive effects can be observed in *in vitro* systems directly exposed to high concentrations of iron ions, likely causing marked increases of free radicals. However, such results might be relevant to an *in vivo* hazard only in cases of evident iron overload, which, in turn, could also have other toxicological effects. The physiological mechanisms in the body normally ensure that iron overload does not occur and that iron is not present as potentially toxic free ions but bound to proteins (such as transferrin, ferritin, hemosiderin) (EVM, 2003; Ferro et al., 2012; Lawen and Lane, 2013).

Besides supplements, the main dietary sources of iron are meat and liver from ruminants; milk and dairy products are particularly low in iron content. The average intake in eight EU countries ranged from 10 to 22 mg/day; the 97.5th percentile ranged from 17 to 41 mg/day, with an extreme value of 72 mg/day in women from Ireland (EFSA, 2004).

3.2.4. Conclusions of safety for consumers

There is no evidence that iron from chelate of amino acids would lead to a significantly increased iron concentration in edible tissues and products of animal origin. Any increase resulting from the use of iron chelate of amino acids compared with the historical practice of supplementing feedingstuffs with inorganic iron compounds is not to be expected.

Since the supplementation of animal feed with iron-containing compounds has not essentially changed during the last decades, it is reasonable to assume that the iron levels in food of animal origin used in exposure scenarios originated from animals fed iron-supplemented diets. Since iron chelate of amino acids will be used as a substitute for other iron compounds, its use in animal nutrition would not modify consumer exposure to iron.

The FEEDAP Panel notes that a European UL for iron has not been established and that susceptible individuals may be more vulnerable to iron overload. However, the FEEDAP Panel does not see any concern for consumer safety resulting from the use of iron chelate of amino acids, hydrate, in animal nutrition provided the maximum contents in feedingstuffs are respected.

3.3. Safety for the users/workers

No specific studies have been provided by the applicant. Some iron compounds are recognised as skin and respiratory irritants. The additive should therefore be considered as a skin, eye and respiratory irritant and, owing to its residual peptide component, as a skin/respiratory sensitiser.

The particle size distribution of four batches of a granular powder form of the additive showed the virtual absence of particles below 75 µm. Dusting potential was not measured and therefore user exposure by inhalation cannot be fully characterised. The FEEDAP Panel notes that inhalation of dust from this product should be considered hazardous and should be avoided.

3.4. Safety for the environment

Based on the calculation method provided in the technical guidance for assessing the safety of feed additives for the environment (EFSA, 2008b), the greatest increase of iron in soil is around 13.5 mg/kg after a 1-year application of manure from pigs for fattening assuming that 100 % of the dose will be excreted. The iron content of soils is typically in the range of 5000 to 50000 mg/kg.

Iron concentrations in surface water are highly variable; Neal and Robson (2000) quote values of <10 to over 1000 µg Fe/L for United Kingdom river water; Lahermo et al. (1996) give iron values ranging from <5 to over 3600 µg/L for Finnish stream water. Anthropogenic sources of iron include the iron and steel industry, sewage and dust from iron mining (Reimann and de Caritat, 1998).

Considering the high background concentration of iron in soil and water, the supplementation of feed with iron chelate of amino acids, hydrate, is not expected to pose an environmental risk.

4. Efficacy

Ferrous chelate of amino acids, hydrate, is a currently authorised compound of the trace element iron (Fe). According to Commission Regulation (EC) No 429/2008,[28] no efficacy studies are required for compounds of trace elements already authorised as feed additives. The product has been used for many years in the EU and has proven to be an available source of iron under practical conditions in different species.

The use of iron chelate of amino acids, hydrate, in animal nutrition is well documented in the scientific literature (e.g. Brady et al., 1978; Henry and Miller, 1995; Wei et al. 2005). It is recognised as an efficacious source of iron in meeting animal requirements. The applicant submitted four published papers (one in laying hens and three in pigs). The study in laying hens (Park et al., 2004) was not considered since product identity to that under application could not be established; the experiment with pigs for fattening (Apple et al., 2007) also could not be considered owing to weaknesses in design and reporting. The remaining two studies in piglets (Yu et al., 2000; Grela et al., 2005) support the general knowledge that iron from iron chelate of amino acids, hydrate, is a source of bioavailable dietary iron.

The FEEDAP Panel concludes that iron chelate of amino acids, hydrate, is an effective source of iron for all animal species and categories.

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[29] and Good Manufacturing Practice.

[28] 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.

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

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

The use of iron chelate of amino acids, hydrate, as source of iron, is considered safe for all animal species/categories when used up to the currently authorised maximum content of total iron in complete feed (ovine: 500 mg Fe/kg complete feedingstuffs; piglets up to one week before weaning: 250 mg Fe/day; other species: 750 mg Fe/kg complete feedingstuffs), with the exception of bovines and poultry for which the maximum tolerated level is 450 mg/kg complete feed, and for pets, for which the maximum tolerated level is 600 mg/kg complete feed. The FEEDAP Panel is not in the position to derive a maximum safe iron concentration in feed for horses or fish.

Consumption surveys include iron-containing foodstuffs of animal origin. Since the supplementation of animal feed with iron-containing compounds has not essentially changed during the last decades, it is reasonable to assume that the iron levels in food of animal origin used in exposure scenarios originated from animals fed iron-supplemented diets. Since iron chelate of amino acids, hydrate, will be used as a substitute for other iron compounds, its use in animal nutrition would not modify consumer exposure to iron.

The additive should be considered as a skin, eye and respiratory irritant and, owing to its residual peptide component, as a skin/respiratory sensitiser.

Considering the high background concentration of iron in soil and water, the supplementation of feed with iron chelate of amino acids, hydrate, is not expected to pose an environmental risk.

Iron chelate of amino acids, hydrate, is an effective source of iron for all animal species and categories.

RECOMMENDATIONS

In the current legislation the name of the additive is 'Ferrous chelate of amino acids, hydrate'. The FEEDAP Panel recommends to change the name to 'Iron chelate of amino acids, hydrate'.

The description of the product proposed by the applicant should be amended as follows: Fe $(x)_{1-3} \cdot nH_2O$ (x= anion of any amino acid derived from acid hydrolysed soya protein). At least 90 % of the molecules should have a molecular weight not exceeding 1500 Dalton.

Since the iron compounds are not available for control, and valuable information to the user of the product can be given only for the final product, the FEEDAP Panel proposes to evaluate the possibility of introducing the formulated product in the register of feed additives.

Based on considerations of animal safety, the FEEDAP Panel recommends the modification of some of the currently authorised maximum iron contents in complete feed as follows:

- bovines and poultry: 450 mg Fe/kg complete feed
- pets: 600 mg Fe/kg complete feed

GENERAL REMARKS

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

DOCUMENTATION PROVIDED TO EFSA

1. Dossier Iron Amino Acid Chelate, Hydrate (Availa® Fe) for all animal species. July 2010. Submitted by Zinpro Animal Nutrition Inc.

2. Dossier Iron Amino Acid Chelate, Hydrate (Availa® Fe) for all animal species. Supplementary information. January 2013. Submitted by Zinpro Animal Nutrition Inc.

3. Evaluation report of the European Union Reference Laboratory for Feed Additives on the methods(s) of analysis for Iron (E1).

4. Comments from Member States received through the ScienceNet.

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APPENDICES

APPENDIX A

Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Iron (E1)[1]

In the current application authorisation is sought under articles 4(1) and 10(2) for *ferrous chelate of glycine hydrate*[2], *ferrous/iron chelate of amino acids hydrate*[2,3], *ferrous fumarate*[2], *ferric oxide*[4], *ferric chloride hexahydrate*[2], *ferrous sulfate monohydrate*[2,5], *ferrous sulfate heptahydrate*[2,6], *ferrous carbonate*[2,7] 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 *ferrous chelate of glycine hydrate* is a green-gray free-flowing powder with a minimum content of 17 % *total iron*, *ferrous/iron chelate of amino acids hydrate* is a brown free-flowing powder with a minimum content of 10 % *total iron*, *ferrous fumarate* is white reddish powder with a minimum content of 30 % *total iron*, *ferric oxide* is a red brown powder with a minimum content of 56% *total iron*, *ferric chloride hexahydrate* is a yellow brown solid aggregate with a minimum content of 59 % *total iron*, *ferrous sulfate monohydrate* consists of beige to gray free-flowing granules with a minimum content of 29 % *total iron*, *ferrous sulfate heptahydrate* is a blue-green crystalline powder with a minimum content of 18 % *total iron* and *ferrous carbonate* is a brown powder with a minimum content of 37 % *total iron*. These *feed additives* are intended to be mixed into *premixtures*, *feedingstuffs* and/or *water*(*). The Applicants suggested maximum levels ranging from 250 to 1250 mg *total iron* /kg *feedingstuffs* and from 100 to 2273 mg *total iron* /L *water*, similar to limits set in the previous regulations.

For the identification and quantification of the inorganic iron compounds (i.e. *ferrous fumarate*, *ferric chloride hexahydrate* and *ferrous sulphate mono* and *heptahydrate*) in the *feed additive*, the EURL recommends for official control the relevant titrimetric methods described in the European Pharmacopoeia Monographs 0083, 0902 and 1515. As for the identification of *ferrous carbonate* and *ferric oxide* the EURL recommends using X-ray diffraction.

For the determination of *ferric oxide* (also know as *iron oxide red)* in the *feed additive* the internationally recognised FAO JECFA monograph for food additives is recommended by Commission Directive 2008/128/EC, laying down specific purity criteria concerning colours for use in foodstuffs. Identification is based on solubility in solvents, while quantification is based on digestion and iodometric titration.

For the quantification of "amino" content in the amino iron chelates (i.e. *ferrous chelate of glycine hydrate* and *ferrous/iron chelate amino acids hydrate*), the Applicant 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.

[1] The full report is available on the EURL website. http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-SANCO-Iron.pdf
[2] FAD-2010-0095.
[3] FAD-2010-0068.
[4] FAD-2010-0236.
[5] FAD-2010-0295.
[6] FAD-2010-0296.
[7] FAD-2010-0380.

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 *ferric oxide, ferric chloride hexahydrate, ferrous carbonate and ferrous sulfate mono* and *heptahydrate*.

For the *quantification* of total iron 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 16 % 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 iron in *feedingstuffs*, in which precisions ranging from 1.0 to 9.5 % were reported.

For the quantification of total iron in *water* the Applicant (FAD-2010-0095) submitted the ring trial validated method EN ISO 11885, based on ICP-AES. The following performance characteristics were reported: a relative standard deviation for *repeatability* (RSDr) ranging from 1.5 to 2.4 %, a relative standard deviation for *reproducibility* (RSDR) ranging from 4.9 to 5.9 %, 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 iron 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

(*) not for *ferric oxide, ferrous carbonate* and *ferrous chelate of amino acids hydrate*.

APPENDIX B

List of Risk Assessment Reports on iron and iron compounds

In addition to the reports cited in the Background section, risk assessments from other EU bodies and Institutions have been carried out.

1. EU risk assessment reports

Food Standard Agency Risk Assessment iron. http://www.food.gov.uk/multimedia/pdfs/evm_iron.pdf)

Scientific Advisory Committee on Nutrition Assessment iron. (http://www.sacn.gov.uk/pdfs/sacn_iron_and_health_report_web.pdf)

2. EFSA-ANS Panel opinions

Iron (II) taurate, magnesium taurate and magnesium acetyl taurate as sources of iron or magnesium added for nutritional purposes in food supplements—Scientific Opinion of the Panel on Food Additives and Nutrient Sources added to Food. (http://www.efsa.europa.eu/en/efsajournal/doc/947.pdf)

Ferrous phosphate added for nutritional purposes to food supplements—Scientific Opinion of the Panel on Food Additives and Nutrient Sources added to Food. (http://www.efsa.europa.eu/en/efsajournal/doc/951.pdf)

Chromium(III)-, iron(II)- and selenium-humic acid/fulvic acid chelate and supplemented humifulvate 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/1147.pdf)

Orotic acid salts as sources of orotic acid and various minerals 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/1187.pdf)

Scientific Opinion on the use of ferric sodium EDTA as a source of iron added for nutritional purposes to foods for the general population (including food supplements) and to foods for particular nutritional uses—EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) (http://www.efsa.europa.eu/en/efsajournal/doc/1414.pdf).

Scientific Opinion on the safety of ferrous ammonium phosphate as a source of iron added for nutritional purposes to foods for the general population (including food supplements) and to foods for particular nutritional uses—EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) (http://www.efsa.europa.eu/en/efsajournal/doc/1584.pdf).

Scientific Opinion on the safety of heme iron (blood peptonates) for the proposed uses as a source of iron added for nutritional purposes to foods for the general population, including food supplements—EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) (http://www.efsa.europa.eu/en/efsajournal/doc/1585.pdf)

3. EFSA-CEF Panel opinions

Scientific Opinion Flavouring Group Evaluation 42: Ion containing organic substances from chemical group 30 (http://www.efsa.europa.eu/en/efsajournal/doc/1191.pdf).

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).

4. EFSA-AFC Panel opinions

Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request from the Commission related to a 6th list of substances for food contact materials (http://www.efsa.europa.eu/en/efsajournal/doc/161.pdf)

Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to Ferrous bisglycinate as a source of iron for use in the manufacturing of foods and in food supplements. (http://www.efsa.europa.eu/en/efsajournal/doc/299.pdf)

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

Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request related to a 18th list of substances for food contact materials (http://www.efsa.europa.eu/en/efsajournal/doc/628.pdf).

5. EFSA-NDA Panel opinions

Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to the Tolerable Upper Intake Level of Iron. (http://www.efsa.europa.eu/en/efsajournal/doc/125.pdf)

Lactobacillus plantarum 299v (DSM 9843) and improve iron absorption Scientific substantiation of a health claim related to *Lactobacillus plantarum* 299v (DSM 9843) and improve iron absorption pursuant to Article 13(5) of Regulation (EC) No 1924/2006—Scientific Opinion of the Panel on Dietetic Products, Nutrition and Allergies. (http://www.efsa.europa.eu/en/efsajournal/doc/999.pdf)

Scientific Opinion on the substantiation of health claims related to iron and formation of red blood cells and haemoglobin (ID 249, ID 1589), oxygen transport (ID 250, ID 254, ID 256), energy-yielding metabolism (ID 251, ID 1589), function of the immune system (ID 252, ID 259), cognitive function (ID 253) and cell division (ID 368) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. (http://www.efsa.europa.eu/en/efsajournal/doc/1215.pdf)

Scientific Opinion on the substantiation of health claims related to vitamin A and cell differentiation (ID 14), function of the immune system (ID 14), maintenance of skin and mucous membranes (ID 15, 17), maintenance of vision (ID 16), maintenance of bone (ID 13, 17), maintenance of teeth (ID 13, 17), maintenance of hair (ID 17), maintenance of nails (ID 17), metabolism of iron (ID 206), and protection of DNA, proteins and lipids from oxidative damage (ID 209) pursuant to Article 13(1) of Regulation (EC) No 1924/2006—EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). (http://www.efsa.europa.eu/en/efsajournal/doc/1221.pdf)

Scientific Opinion on the substantiation of health claims related to vitamin B6 and protein and glycogen metabolism (ID 65, 70, 71), function of the nervous system (ID 66), red blood cell formation (ID 67, 72, 186), function of the immune system (ID 68), regulation of hormonal activity (ID 69) and mental performance (ID 185) pursuant to Article 13(1) of Regulation (EC) No 1924/2006—EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). (http://www.efsa.europa.eu/en/efsajournal/doc/1225.pdf)

Scientific Opinion on the substantiation of health claims related to vitamin C and protection of DNA, proteins and lipids from oxidative damage (ID 129, 138, 143, 148), antioxidant function of lutein (ID 146), maintenance of vision (ID 141, 142), collagen formation (ID 130, 131, 136, 137, 149), function of the nervous system (ID 133), function of the immune system (ID 134), function of the immune system during and after extreme physical exercise (ID 144), non-haem iron absorption (ID 132, 147), energy-yielding metabolism (ID 135), and relief in case of irritation in the upper

respiratory tract (ID 1714, 1715) pursuant to Article 13(1) of Regulation (EC) No 1924/2006—EFSA Panel on Dietetic Products, Nutrition and Allergies(NDA). (http://www.efsa.europa.eu/en/efsajournal/doc/1226.pdf)

Scientific Opinion on the Substantiation of a health claim related to Iron and cognitive development of children pursuant to Article 14 of Regulation (EC) No 1924/2006—EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) (http://www.efsa.europa.eu/en/efsajournal/doc/1360.pdf).

Scientific Opinion on the substantiation of health claims related to iron and formation of red blood cells and haemoglobin (ID 374, 2889), oxygen transport (ID 255), contribution to normal energy-yielding metabolism (ID 255), reduction of tiredness and fatigue (ID 255, 374, 2889), biotransformation of xenobiotic substances (ID 258), and "activity of heart, liver and muscles" (ID 397) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. (http://www.efsa.europa.eu/en/efsajournal/doc/1740.pdf)

Scientific Opinion on the substantiation of health claims related to various food(s)/food constituent(s) and improved bioavailability of nutrients (ID 384, 1728, 1752, 1755), energy and nutrient supply (ID 403, 413, 457, 487, 667, 1675, 1710, 2901, 4496) and presence of a nutrient in the human body (ID 720) pursuant to Article 13(1) of Regulation (EC) No 1924/2006—EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). (http://www.efsa.europa.eu/en/efsajournal/doc/1743.pdf)

Scientific Opinion on the substantiation of health claims related to riboflavin (vitamin B2) and contribution to normal energy-yielding metabolism (ID 29, 35, 36, 42), contribution to normal metabolism of iron (ID 30, 37), maintenance of normal skin and mucous membranes (ID 31, 33), contribution to normal psychological functions (ID 32), maintenance of normal bone (ID 33), maintenance of normal teeth (ID 33), maintenance of normal hair (ID 33), maintenance of normal nails (ID 33), maintenance of normal vision (ID 39), maintenance of normal red blood cells (ID 40), reduction of tiredness and fatigue (ID 41), protection of DNA, proteins and lipids from oxidative damage (ID 207), and maintenance of the normal function of the nervous system (ID 213) pursuant to Article 13(1) of Regulation (EC) No 1924/2006—EFSA Panel on Dietetic Products, Nutrition and Allergies(NDA). (http://www.efsa.europa.eu/en/efsajournal/doc/1814.pdf

Scientific Opinion on the substantiation of health claims related to meat or fish and the improvement of non-haem iron absorption (ID 1223) pursuant to Article 13(1) of Regulation (EC) No 1924/2006—EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) (http://www.efsa.europa.eu/en/efsajournal/doc/2040.pdf)

Scientific Opinion on the substantiation of a health claim related to iron and maintenance of normal hair growth pursuant to Article 13(5) of Regulation (EC) No 1924/2006—EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). (http://www.efsa.europa.eu/en/efsajournal/doc/2602.pdf)

Scientific Opinion on bovine lactoferrin—EFSA Panel on Dietetic Products, Nutrition and Allergies (http://www.efsa.europa.eu/en/efsajournal/doc/2701.pdf)

APPENDIX C

List of authorisations of iron compounds other than feed additive

The following iron compounds are authorised for use in food (Regulation (EC) No 1170/2009)[8]: ferrous L-pidolate, ferrous phosphate, iron (II) taurate which may be used in the manufacture of food supplements; ferrous carbonate, ferrous citrate, ferrous ammonium citrate, ferrous gluconate, ferrous fumarate, ferric sodium diphosphate, ferrous lactate, ferrous sulphate, ferric diphosphate (ferric pyrophosphate), ferric saccharate, elemental iron (carbonyl + electrolytic + hydrogen reduced) and ferrous bisglycinate which may be used in the manufacture of food supplements and may be added to food. Ferrous gluconate (579) and ferrous lactate (E585) are authorised as food additives for use in olives darkened by oxidation at the maximum level of 150 mg/g as Fe (European Parliament and Council Directive No 95/2/EC).[9]

The following iron compounds can be used for the manufacturing of dietetic foods (Commission Regulation (EC) No 953/2009)[10]: ferrous carbonate, ferrous citrate, ferrous ammonium citrate, ferrous gluconate, ferrous fumarate, ferric sodium diphosphate, ferrous lactate, ferrous sulphate, ferric diphosphate (ferric pyrophosphate), ferric saccharate, elemental iron (carbonyl + electrolytic + hydrogen reduced), ferrous bisglycinate and ferrous L-pidolate.

The following iron 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)[11]: ferrous citrate, ferrous ammonium citrate, ferrous gluconate, ferrous lactate, ferrous sulphate, ferrous fumarate, ferric diphosphate (ferric pyrophosphate), elemental iron (carbonyl + electrolytic + hydrogen reduced), ferric saccharate, sodium ferric diphosphate and ferrous carbonate.

Regarding pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin, the following iron compounds are listed in Table 1 of the Annex of Regulation 37/2010[12] as *Allowed substances, no MRL required*: iron ammonium citrate, iron dextran, iron dichloride, iron fumarate, iron glucoheptonate and iron sulphate.

The following iron compounds are listed in Annex of Commission Implementing Regulation (EU) No 540/2011[13] as "Active substances approved for use in plant protection products": iron sulphate, iron (II) sulphate anhydrous, iron (II) sulphate monohydrate, iron (II) sulphate heptahydrate (iron (II) sulphate) and ferric phosphate.

The following type of fertilisers for iron as *Fertilisers containing only one micro-nutrient* are listed in Annex I of Regulation (EC) No 2003/2003 of the European Parliament and of the Council:[14] (a) iron salt (chemically obtained product containing a mineral iron salt as its essential ingredient); (b) iron chelate (water soluble product obtained by chemical reaction of iron with chelating agents mentioned in the list of Annex I chapter E.3 which are sodium, potassium or ammonium acids or salts of EDTA,

[8] 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.

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

[10] 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.

[11] 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.

[12] 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.

[13] 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.

[14] 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.

DTPA, EDDHA, HEEDTA, EDDHMA, EDDCHA) and iron fertiliser solution (product obtained by dissolving types (a) and/or one of the type (b) in water).

The following iron compounds can be used for cosmetic purposes (Regulation (EC) No 1223/2009 of the European Parliament and of the Council[15]): iron oxide, iron oxide red, iron oxide yellow, iron oxide black, ferric ammonium ferrocyanide, aluminium silicate coloured with ferric oxide and natural hydrated aluminium silicate, $Al_2O_3 \cdot 2SiO_2 \cdot 2H_2O$ with iron carbonates or ferric hydroxide impurities.

According to the Annex of Regulation (EC) No 432/2012,[16] the following health claims can be made only for food which is at least a source of iron 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:[17] iron contributes to normal cognitive function, iron contributes to normal energy-yielding metabolism, iron contributes to normal formation of red blood cells and haemoglobin, iron contributes to normal oxygen transport in the body, iron contributes to the normal function of the immune system, iron contributes to the reduction of tiredness and fatigue and iron has a role in the process of cell division.

[15] 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.

[16] 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.

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

APPENDIX D

Iron content in animal tissues and products. Food Composition Tables

Species/category	Liver (mg Fe/kg)	Kidney (mg Fe/kg)	Muscle (mg Fe/kg)	Egg (mg Fe/kg)	Milk (mg Fe/kg)	Reference
SWINE						
Pigs		33	5.5-7.1			(1)
	180*		12-17			(2)
	170 (150-310)	73 (53-150)	10 (10-11)			(3)
RUMINANTS						
Veal		60.8	14.5-16			(1)
			12			(2)
	55 (57-93)	120 (79-150)	21 (15-26)			(3)
Cattle			16-24.7			(1)
	88	80	13-19			(2)
	69 (44-72)	110 (65-150)	21 (17-23)			(3)
Dairy cattle					0.6	(1)
					1	(2)
					0.46 (0.3-0.7)	(3)
Lamb			12-22			(1)
			17-20			(2)
Sheep	126				1	(2)
	120 (120-130)	75 (41-92)	18 (15-23)		0.58 (0.51-1.0)	(3)
Goat					1	(2)
					0.41 (0.36-0.75)	(3)
POULTRY						
Chickens	90.15		18			(1)
			6-14			(2)
	74		7.3 (6-20)			(3)
Laying hens	70.6		8-10.1	20		(1)
				55 [yolk]		
				15		(2)
				18		(3)
				72 (51-120) [yolk]		
Hens			16			(2)
Turkey			7.7			(1)
			9-10			(2)
Duck	300.5		12	38.5		(1)
			13			(2)
Goose			25	36.4		(1)
	290.6		18			(2)
RABBITS			10			(2)
			27 (18-60)			(3)
HORSES			35			(1)
	90		39			(2)
			49			(3)
FISH						
Cod			23			(2)
Herring			13			(1)
			9			(2)
			9.8 (5.9-10)			(3)
Mackerel			12			(2)
			12 (8-14)			(3)
Eel			10			(2)
Trout			7			(1)
			20**			(2)
			4.1			(3)
Tuna			15			(1)

	13	(2)
Carp	10	(2)
	7 (6-13)	(3)
Salmon	8	(1)
	7	(2)
	5.8 (4-15)	(3)

(*) Data are reported as from the reference, i.e. as a single figure, as average (and range) or as a range.

(**) Farmed trouts

References

(1) Danish Food Consumption Databank – Ed. 7.01. National Food Institute—Technical University of Denmark. http://www.foodcomp.dk/v7/fcdb_foodnutrlist.asp?CompId=0061

(2) Database of INRAN - Italian National Institute for Research on Foods and Nutrition. http://www.inran.it/646/tabelle_di_composizione_degli_alimenti.html

(3) Souci SW, Fachmann W and Kraut H, 2008. Food composition and nutrition tables. 7th edn. MedPharm Scientific Publisher, Stuttgart, Germany; and CRC Press, Taylor and Francis Group, LLC, Boca Raton, FL, USA.