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Safety and efficacy of a feed additive consisting of iron (II)-betaine complex for all animal species (Biochem Zusatzstoffe Handels- und Produktionsges. mbH)

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Abstract

Following a request from the European Commission, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) was asked to deliver a scientific opinion on the safety and efficacy of iron(II)-betaine complex as a nutritional feed additive for all animal species. Based on the results of a tolerance study carried out in chickens the FEEDAP Panel concluded that the additive is safe for chickens for fattening when used up to the current maximum authorised levels of iron in feed; this conclusion was extrapolated to all animal species and categories at the respective maximum iron levels in complete feed authorised in the European Union. The FEEDAP Panel concluded that the use of the iron(II)-betaine complex in animal nutrition at the maximum iron levels authorised for the animal species poses no concern to the safety of consumers. The additive is not a skin irritant, but it is an irritant to the eyes. Due to the traces of nickel, the additive is considered to be a respiratory and skin sensitiser. Regarding the safety for the environment, the use of the additive in feed for terrestrial animals, land-based and sea cages aquaculture is considered safe under the proposed conditions of use. Based on the deposition of iron in edible tissues/organs in chickens for fattening, the FEEDAP Panel concluded that the additive is a source of bioavailable iron, comparable to the standard inorganic iron source, and therefore, the additive is efficacious in meeting the birds iron requirements. This conclusion can be extrapolated to all animal species and categories.

Keywords: nutritional additives, compounds of trace elements, iron, iron complex of betaine, safety, efficacy

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1. Introduction

1.1. Background and Terms of Reference

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

The European Commission received a request from Biochem Zusatzstoffe Handels- und Produktionsges mbH[2] for the authorisation of the additive consisting of iron(II)-betaine complex, when used as a feed additive for all animal species (category: nutritional additives; functional group: compounds of trace elements).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 8 February 2023.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the feed additive consisting of iron(II)-betaine complex, when used under the proposed conditions of use (see **Section 3.1.5**).

1.2. Additional information

The additive, iron(II)-betaine complex is intended to be used as a source of iron in all animal species. It has not been previously authorised as a feed additive in the European Union.

2. Data and Methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[3] in support of the authorisation request for the use of iron(II)-betaine complex as a feed additive. The dossier was received on 7/10/2022 and the general information and supporting documentation is available at https://open.efsa.europa.eu/questions/EFSA-Q-2022-00624.

The confidential version of the technical dossier was subject to a target consultation of the interested Member States from 8 February 2023 to 8 May 2023 for which the received comments were considered for the assessment.

In accordance with Article 38 of the Regulation (EC) No 178/2002[4] and taking into account the protection of confidential information and of personal data in accordance with Articles 39 to 39e of the same Regulation, and of the Decision of EFSA's Executive Director laying down practical arrangements concerning transparency and confidentiality,[5] a non-confidential version of the dossier has been published on Open.EFSA.

According to Article 32c(2) of Regulation (EC) No 178/2002 and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations,[5] EFSA carried out a public consultation on the non-confidential version of the technical dossier from 17 July 2023 to 7 August 2023 for which no comments were received.

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the council of 22 September 2003 on the additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] Biochem Zusatzstoffe Handels- und Produktionsges mbH, Küstermeyerstr. 16, 49393 Lohne, Germany.
[3] Dossier reference: FEED-2022-9491.
[4] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–48.
[5] Decision available online: https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the iron(II)-betaine complex in animal feed.[6]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of iron(II)-betaine complex is in line with the principles laid down in Regulation (EC) No 429/2008[7] and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017a), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018), Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019).

3. Assessment

The additive under assessment is iron(II)-betaine complex. The additive corresponds to the active compound. It is intended for use in feed as a nutritional additive (functional group: compounds of trace elements) for all animal species and categories.

3.1. Characterisation

3.1.1. Characterisation of the additive

The additive is a complex defined by the applicant with a chemical formula $[Fe(H_2O)_2((CH_3)_3NCH_2COO)(SO_4)]_n$, corresponding to a molecular weight of 305.06 g/mol for the repeat unit. International Union of Pure and Applied Chemistry (IUPAC) chemical name is catena-[diaqua-sulfato-μ2-(trimethylammonio)acetato-iron(II)], Chemical Abstracts Service (CAS) number or EC number are unavailable.

Proof of complex formation between iron, betaine and sulfate was provided by using X-ray powder diffraction.[8] X-ray patterns of the starting materials [redacted] and of one batch of iron(II)-betaine complex were measured. Results showed that the starting materials were no longer present and that the conversion into a new product appeared to be complete.

The Panel notes that the data also indicate that the additive is a coordination polymer, [redacted][9]

Based on the manufacturer's specification, iron(II)-betaine complex contains minimum 14% of iron, minimum 36% of betaine, sulfur content 9–12% (equivalent to 27–36% of sulfate, calculated from reported sulfur) and a maximum 5% of moisture.[10]

Analytical data to confirm the specifications were provided for 10 batches of the additive,[11] showing the following average values: 15.5% (range 14.9–16.4%) iron, 37.8% (range 36.8–39.1%) betaine, 10.1% (range 9.6–10.4%) sulfur and 0.8% (range 0.5–1.1%) water. Content of iron, sulfate (recalculated from sulfur), betaine, crystalised water, trace of manganese,[12] ash insoluble in HCl were tested in five batches and summed up to 99.7% on average.[13]

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[6] Evaluation report received on 19/04/2023 and available on the EU Science Hub https://joint-research-centre.ec.europa.eu/eurl-fa-eurl-feed-additives/eurl-fa-authorisation/eurl-fa-evaluation-reports_en

[7] 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.

[8] Annex II_12.

[9] Annex_II_11.

[10] Standard water (4 h, 103°C).

[11] Annex_II_1 and Annex_II_3.

[12] Residues of manganese may be present due to the cross-contamination from previous test production containing manganese salts.

[13] Annex_II_2.

Three batches of the additive were analysed for undesirable substances.[14] Levels of cadmium, lead, mercury and arsenic were below the limits of quantification (LOQ) of the analytical methods.[15] Fluorine and nickel[16] content in the additive (analysis of three batches)[17] showed an average of < 0.40 mg/kg (LOQ) and 35.7 mg/kg (range 35.2–36.5), respectively. The levels of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/F) and the sum of PCDD/F and dioxin-like polychlorinated biphenyls (PCBs) were 0.16–0.17 ng WHO-PCDD/F-TEQ/kg and 0.30–0.31 ng WHO-PCDD/F-PCB-TEQ/kg, respectively; non-dioxin-like PCBs were in the range of 1.7–5.8 μg/kg.

Levels of mycotoxins aflatoxin B1 and ochratoxin A, analysed in three batches, were below the respective LOQ.[18]

Three batches of the additive were analysed for microbiological contamination.[19] *Salmonella* spp. was not detected in 25 g, and *Enterobacteriaceae* and *Escherichia coli* were < 10^2 colony forming unit (CFU)/g and < 10 CFU/g, respectively.

The FEEDAP Panel considers that the microbial contamination and the amounts of detected impurities, with the exception of nickel, do not raise safety concerns.

3.1.2. Physical properties of the additive

The additive appears as a white-beige powder with a bulk density of 821 kg/m^3 (average of three batches).[20]

Three batches were analysed for dusting potential by the Stauber-Heubach method and for particle size distribution (four replicate measurements/batch) by laser diffraction.[20] The results showed a dusting potential ranging from 390 to 630 mg/m^3 air and 0.1% of particles with diameter < 100 μm, no particles below 10 μm were found.[21] The applicant provided data on the iron content of the dust measured in one sample (four replicate measures); the average iron content was 158.28 g Fe/kg dust (range 149.43–173.91 g Fe/kg).[22]

Solubility was tested in five batches of the additive[23], showing a range between 100 and 110 g/L (freely soluble) in water at 20°C (EFSA Scientific Committee, 2021). Based on the solubility results, there is no need to further characterise the fraction of small particles, including nanoparticles, potentially present in the additive (EFSA Scientific Committee, 2021). The pH (10% water solution) was 2.96; the additive is insoluble in organic solvents.

3.1.3. Manufacturing process

Full details of the manufacturing process for iron(II)-betaine complex are described in the dossier. [redacted]

3.1.4. Stability and homogeneity

For mineral-based additives, stability studies are generally not required. No studies on shelf-life have been provided.

The capacity for homogeneous distribution of the additive was examined in complete feed (meal) and pelleted feed (pelleting temperature 70°C). Ten subsamples of each feed item were tested for

[14] Annex_II_5.
[15] Limit of quantification (LOQ): lead 0.5 mg/kg, mercury 0.02 mg/kg, cadmium 0.20 mg/kg and arsenic 0.50 mg/kg.
[16] The applicant stated that per manufacturer's specification nickel must be below 100 mg/kg in the additive.
[17] Annex_II_6.
[18] Annex II_5. LOQ aflatoxin B1 = 0.3 μg/kg and ochratoxin A = 0.5 μg/kg.
[19] Annex II_4.
[20] Annex_II_7.
[21] Annex_II_8.
[22] Annex_II_9.
[23] Annex_II_Add Info_Certificate_Water solubility.

iron. The mean concentration of iron was 283.3 mg/kg in meal[24] and 288.7 mg/kg in pelleted feed,[25] with corresponding coefficients of variation (CVs) of 8.3% and 6.3%, respectively.

3.1.5. Conditions of use

The additive is intended to be used in feed – directly or via a premixture or complementary feed – for all animal species up to a maximum authorised total iron content in complete feed[26]: 500 mg/kg for ovine, 450 mg/kg for bovine and poultry, 250 mg/kg for piglets until 1 week before weaning, 600 mg/kg for pets and 750 mg/kg for other species.

3.2. Safety

The Panel notes that the additive is a coordination polymer formed by iron, sulfate and betaine. Based on the data available uncertainty remains on the size of the coordination polymer. Although there is uncertainty on how it would affect the dissociation rate, considering the nature of the coordination bonds between iron(II), betaine and sulfate ions, the FEEDAP Panel considers it likely that under physiological conditions in the animal gastrointestinal tract the additive dissociates into its components.

The safety of betaine and iron sulfate, when used as feed additives, has already been assessed and established by EFSA FEEDAP Panel (2012, 2013, 2014, 2016).

3.2.1. Safety for the target species

The applicant provided a combined tolerance and efficacy study with iron(II)-betaine complex in chickens for fattening with a duration of 35 days.[27] A total of 840 one-day-old male chickens (Ross 308) were penned in groups of 20 birds and randomly allocated to seven experimental diets (representing six replicates per treatment). Basal (starter, grower and finisher) diets based on maize and soybean meal were either not supplemented (control group, estimated content of iron was 130 mg/kg feed) or supplemented with iron to reach the intended concentration of 250, 450, 750 mg iron/kg complete feed of one of the two sources considered (iron sulfate monohydrate or iron(II)-betaine complex), for a total of seven experimental diets. The details on the iron content, intended and analysed, are provided in Table 1. The diets were offered to birds as mash (starter, grower and finisher) at feed presentations on an *ad libitum* basis for a total of 35 days.

Table 1: Iron content in the experimental diets offered to the experimental groups

Treatment	Source of Iron	Total Fe (mg/kg)			
		Intended	Analysed		
			Starter	Grower	Finisher
T1	None	130	181	181	190
T2	Iron sulfate monohydrate	250	239	240	264
T3		450	441	404	392
T4		750	683	698	878
T5	Iron(II)-betaine complex	250	245	253	288
T6		450	467	488	480
T7		750	766	759	885

[24] Annex II_15.
[25] Annex II_16.
[26] Commission Implementing Regulation (EU) 2017/2330 of 14 December 2017 concerning the authorisation of Iron(II) carbonate, Iron(III) chloride hexahydrate, Iron(II) sulfate monohydrate, Iron(II) sulfate heptahydrate, Iron(II) fumarate, Iron (II) chelate of amino acids hydrate, Iron(II) chelate of protein hydrolysates and Iron(II) chelate of glycine hydrate as feed additives for all animal species and of Iron dextran as feed additive for piglets and amending Regulations (EC) No 1334/2003 and (EC) No 479/2006.
[27] Technical dossier text-3.1 Safety of use of the additive for the target species.

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Mortality and general health were monitored throughout the study. Body weight (bw) and feed intake were measured on days 1, 10, 21 and 35 and feed to gain ratio was calculated for the corresponding periods. At the end of the study (day 35), two birds per replicate were sampled for blood collection to analyse for haematology[28] and biochemistry.[29] Necropsy, including weighing of organs,[30] was done in the same two birds per pen on the same date. Samples from kidneys, liver, muscle and skin fat were collected from two birds per pen in order to analyse the content of iron (see Section 3.2.2.1).

The data were statistically analysed with a one-way analysis of variance considering the pen as the experimental unit for the performance, and the animal for the rest of the parameters. Group means were compared with Tukey test to account for multiplicity. The significance level was established at 0.05.

The results on the mortality and zootechnical performance are presented in Table 2. Mortality was 1.4% in the overall study and no difference was found between groups.

Table 2: Effect of iron(II)-betaine complex on performance parameters and mortality in chickens for fattening after 35 days

Treatment	Source of added iron	Intended total iron (mg/kg)	Average daily feed intake (g)	Body weight (g)	Feed to gain ratio	Mortality and culling % (n)
T1	None	130	93.1	2,162	1.55^{ab}	2.5 (3)
T2	Iron sulfate monohydrate	250	94.3	2,208	1.54^{ab}	0.8 (1)
T3		450	92.3	2,187	1.52^{ab}	2.5 (3)
T4		750	90.2	2,095	1.55^{ab}	1.7 (2)
T5	Iron(II)-betaine complex	250	93.7	2,137	1.57^{b}	0.8 (1)
T6		450	92.7	2,161	1.54^{ab}	0.8 (1)
T7		750	92.2	2,227	1.48^{a}	0.8 (1)

a,b: Means in a column not sharing a common letter are statistically different ($p < 0.05$).

When considering the performance parameters, no significant differences among groups were observed for the bw of the birds at day 35 (average across treatments: bw = 2,168 g).

At day 35, statistical differences were observed for fibrinogen and lymphocytes. Fibrinogen was statistically lower in the groups receiving the overdose of iron sulfate and the maximum proposed use level of iron(II)-betaine complex compared to the control. Lymphocytes were statistically higher at the maximum proposed level of iron(II)-betaine complex compared to the control. No significant differences between the control and experimental groups were observed for the biochemical parameters except for the alkaline phosphatase, where a significant reduction was observed in the iron-supplemented groups (except the overdose iron(II)-betaine complex). All the differences were within the physiological values for this species.

The tolerance study indicates that iron(II)-betaine complex is safe up to 750 mg Fe/kg feed in chickens for fattening.

3.2.1.1. Conclusions on safety for the target species

Based on the results of the tolerance study, the FEEDAP Panel concludes that the additive is safe for chickens for fattening up to the maximum recommended level. This conclusion can be extrapolated to all animal species and categories provided that the maximum authorised levels in the EU for total iron in feed are not exceeded.

[28] Erythrocytes, Haemoglobin, Haematocrit, MCV (spell), MCH (spell), MCHC (spell), Leukocytes, Lymphocytes, Monocytes, Eosinophils, Heterophiles, Thrombocyte count, Fibrinogen and Prothrombin time.

[29] Bilirubin total, Cholesterol, Protein total, Albumin, Globulin, Glucose, Urea, Calcium, Chloride, Potassium, Magnesium, Sodium, Inorg. Phosphate, Creatinine, Serum-Amyloid A, α-Amylase, ALT (Alanine aminotransferase), ALP (Alkaline phosphatase), AST (Aspartate aminotransferase), CK (Creatine kinase), y-GT (Gamma-Glutamyl transferase), GLDH (Glutamate dehydrogenase), LDH (Lactate dehydrogenase).

[30] Liver, kidney, spleen, adrenal gland, lungs, stomach, pancreas, ileum, colon, caecum, thymus, thyroid gland, heart and testes.

3.2.2. Safety for the consumer

The sources used for the manufacturing of the iron(II)-betaine complex, are authorised in the EU and their safety has been established. The complex is expected to dissociate under physiological conditions into betaine, iron and sulfate ions. Therefore, the FEEDAP Panel retains that only exposure to iron is of interest concerning consumer safety.

3.2.2.1. Deposition study

The applicant submitted a study on the tolerance of chickens for fattening (see Section 3.2.1.1. This study provided data on iron deposition in tissues and organs (breast muscle, kidney, skin plus fat and liver) of chickens for fattening fed the iron(II)-betaine complex. Samples were taken at day 35 after necropsy, from a total of 84 birds (two birds/pen).

The increase of iron levels in the feed of chickens for fattening up to 750 mg/kg for iron(II)-betaine complex as a source of iron, did not result in an increased deposition of iron in edible tissues. The iron content in the liver was significantly higher in the iron(II)-betaine group corresponding to 750 mg iron/kg feed, compared to the control. No significantly higher deposition of iron was found in birds receiving the maximum authorised level in feed (450 mg iron/kg feed) compared to the control.

Table 3: Analytical results of total iron content in edible tissues (results in fresh matter – two birds per replicate)

Treatment	Iron source	Total Iron mg/kg (intended)	Skin and fat mg Fe/kg	Muscle mg Fe/kg	Liver mg Fe/kg	Kidney mg Fe/kg
T1	None	130	19.7	3.9	160[bc]	73
T2	Iron sulfate monohydrate	250	16.3	4.0	130[c]	90
T3		450	29.1	4.1	248[bc]	82
T4		750	20.7	4.9	336[ab]	89
T5	Iron(II)-betaine complex	250	19.8	4.1	169[bc]	77
T6		450	25.9	4.3	199[bc]	79
T7		750	27.4	5.0	387[a]	80

[a,b,c]: Means in a column not sharing the same superscript are statistically different ($p < 0.05$).

3.2.2.2. Metabolism and toxicology of iron

Metabolism and deposition of iron have been previously reviewed in FEEDAP opinions (see e.g. EFSA FEEDAP Panel, 2016). The transport of iron from enterocytes to blood depends on the iron pool in the liver. Owing to the strong regulation of its intestinal absorption, a high oral intake of iron results in a less than proportional increase in iron deposition. Under physiological conditions, the haemoglobin concentration in the blood closely reflects the amount of iron utilised in the organism and is a biomarker of a potentially deficient iron status. Indeed, ~ 70% of body iron content is present in haemoglobin (EFSA, 2004). When animals are exposed to excessive amounts of iron, it is preferentially deposited in the liver, spleen and bone marrow. With very high doses, iron may be deposited in the heart and kidneys (NRC, 2005). The iron content of milk is highly resistant to changes in the level of dietary iron (NRC, 2005). A number of studies did not give evidence that organic iron sources would significantly influence the iron content of tissues, including muscle or eggs (see EFSA FEEDAP Panel, 2016 and references herein). The safety of iron has been previously evaluated by several authorities (EVM, 2003; EFSA, 2004) and recently evaluated by Ponka et al. (2015). In infants, an acute dose of ~ 20 mg/kg bw, is associated with gastrointestinal irritation, whilst systemic effects do not generally occur at doses < 60 mg/kg bw. In adults, adverse gastrointestinal effects have been reported after short-term oral dosage as low as 50–60 mg daily of supplemental non-haem iron. The EFSA Opinion (EFSA, 2004) assessed a possible tolerable upper intake level (UL) for iron; iron overload with clinical symptoms, including liver cirrhosis, has been reported in individuals receiving long-term, high-dose medical treatment with iron (160–1,200 mg iron/day). The risk of adverse effects from iron overload in the general population, including those heterozygous for hereditary haemochromatosis, is considered to be low; however, the available data are insufficient to establish a UL. In its opinion on reference dietary intakes for iron, the EFSA NDA Panel reiterated that, while a UL is not determined, the risk of systemic iron overload from dietary sources is negligible with normal intestinal function (EFSA NDA Panel, 2015). Chronic iron overload may occur as a result of specific clinical conditions and genetic mutations, but there is no evidence that heterozygotes for haemochromatosis are at an increased risk of iron overload. The Population Reference Intake, calculated as the dietary requirement at the 97.5th percentile, is 11 mg Fe/day for adult men and 16 mg Fe/day for premenopausal women (EFSA NDA Panel, 2015).

3.2.2.3. Assessment of consumers exposure

Based on the residue study in chicken for fattening, the use of iron(II)-betaine complex corresponding to the maximum authorised content of iron in complete feed is not expected to increase the iron content of edible tissues and products, hence increasing the exposure of consumers.

3.2.2.4. Conclusions on safety for the consumer

The use of iron(II)-betaine complex in animal nutrition up to the maximum iron content in complete feed authorised in the EU poses no concern to the safety of consumers.

3.2.3. Safety for the user

3.2.3.1. Effect on respiratory system

No specific inhalation toxicity studies for the product under assessment were provided by the applicant. The dusting potential of iron(II)-betaine complex is up to 630 mg/m^3, therefore, the FEEDAP Panel considers that exposure through inhalation is likely. The iron maximum concentration in the dust was 17.4%. Thus, a maximum concentration of 109.6 mg Fe mg/m^3 may be released by the dust during the handling of the additive.

The nickel content in the additive per specification must be below 100 mg/kg. Considering the worst-case scenario (100 mg Ni/kg), with a dusting potential of 630 mg/m^3 and assuming a similar proportion of nickel in the dust as in the additive, the nickel content in the dust would be up to 0.063 mg Ni/m^3. This value would not exceed the transitional limit value of 0.1 mg Ni/m^3 for the inhalable fraction and 8 h time-weighted average (8 h TWA) exposure established in Directive (EU) 2022/431.[31] However, being nickel present in the additive, it should be considered a respiratory sensitiser.

3.2.3.2. Effect on eyes and skin

The skin irritation potential of iron(II)-betaine complex was investigated in an *in vivo* skin irritation/corrosion study performed with adult female albino rabbits according to OECD TG 404. The results of the study showed that the additive is not a skin irritant (UN GHS 'No Category').[32]

The eye irritation potential of iron(II)-betaine complex was investigated in an *in vivo* eye irritation/corrosion study performed with adult female albino rabbits according to OECD TG 405. The rabbits showed irreversible signs of eye damage (chemosis). Therefore, the additive is classified as an irritant to the eyes (UN-GHS 'Category 1').[33]

No specific studies on the skin sensitisation potential of the additive were submitted. However, due to the presence of nickel in the additive (up to 36.5 mg/kg from three batches), and given its well-known sensitisation potential, the additive is considered a skin and respiratory sensitiser.

3.2.3.3. Conclusions on safety for the user

The FEEDAP Panel considered that exposure by inhalation is likely. Due to the presence of nickel, the additive is considered a dermal and respiratory sensitiser. The additive is irritant to the eyes, but not to the skin.

3.2.4. Safety for the environment

The additive under assessment contains iron, betaine and sulfate. The environmental risk assessment is made assuming that the whole amount of the additive ingested by the animals is excreted.

[31] Directive (EU) 2022/431 of the European Parliament and of the Council of 9 March 2022 amending Directive 2004/37/EC on the protection of workers from the risks related to exposure to carcinogens or mutagens at work. OJ L88, 16.3.2022, pp. 1–14. The limit value of 0.05 mg/m3 for the inhalable fraction, measured as nickel, shall apply from 18 January 2025. Until then a limit value of 0.1 mg/m^3 shall apply.

[32] Annex_III_6.

[33] Annex_III_7.

Sulfates are naturally abundant in the environment and the use of the additive will not result in a substantial change in the natural background concentration under the current conditions of use.[34]

For the betaine moiety, the FEEDAP Panel concluded that it is not of concern since it is naturally present in the environment and its safety has already been assessed EFSA FEEDAP Panel (2013).

Iron is the fourth most abundant element and the second most abundant metal in the Earth's crust, after aluminium. The greatest increase of iron in the soil, due to the use of the additive, is expected to be around 47.9 mg/kg soil dw after a 10-year application of horse manure (750 mg/kg feed). The FOREGS database (2005) reports a 90th percentile value of 66.7 g/kg of Fe_2O_3, corresponding to 46.6 g/kg of iron, representing the natural background concentration of iron in the topsoil. The comparison of the PECsoil plateau (47.9 mg/kg soil dw) with 10% of the natural background concentration (4,660 mg/kg soil dw) indicates that the use of the additive in animal nutrition would have a negligible impact on the environment.

Concerning the use of the additive in aquaculture, the FEEDAP Panel noted that iron is not considered a hazardous substance and its toxicity is very low. Therefore, no concern is expected for the safety of the additive when used in surface water and sea cages at the proposed conditions of use.

3.3. Efficacy

To demonstrate the efficacy of nutritional additives, one study in a single animal species or category, including laboratory animals, is generally considered sufficient for new forms of compounds of trace elements (EFSA FEEDAP Panel, 2018).

3.3.1. Efficacy for chickens for fattening

The applicant provided a combined tolerance/efficacy study in chickens for fattening (see Section 3.2.1). The experimental groups in the study are shown in Table 1. In this trial, the iron concentration in edible tissues/organs was measured (Table 3). The deposition data showed a significant increase of iron concentration in the liver at the highest level tested (750 mg/kg feed as iron (II)-betaine complex) compared to the control (130 mg/kg). Therefore, the Panel concludes that the additive is an efficacious source of iron. This conclusion can be extrapolated to all animal species and categories.

3.4. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[35] and Good Manufacturing Practice.

4. Conclusions

Based on a tolerance study, the FEEDAP Panel concludes that iron(II)-betaine complex is safe for chickens for fattening. This conclusion can be extrapolated to all animal species and categories provided that the maximum authorised levels in the EU for total iron in feed are not exceeded.

The FEEDAP Panel concludes that the use of iron(II)-betaine complex in animal nutrition is of no concern for consumer safety provided that the maximum authorised total iron levels in feed are respected.

Owing to the presence of nickel, the additive is considered a skin and respiratory sensitiser. It is an eye irritant, but it is non-irritant to the skin.

Regarding the safety for the environment, the FEEDAP Panel concludes that no concern is expected regarding the safety of the additive when used in terrestrial animals and aquaculture at the proposed conditions of use.

Based on the deposition of iron in edible tissues/organs in chickens for fattening, the FEEDAP Panel concludes that the additive is efficacious as a source of iron in all animal species and categories. This conclusion can be extrapolated to all animal species and categories.

[34] Based on the data available in FORGES database https://weppi.gtk.fi/publ/foregsatlas/ForegsData.php

[35] 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.

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Abbreviations

ADFI	average daily feed intake
ADG	average daily gain
ADI	acceptable daily intake
bw	body weight
CAS	Chemical Abstracts Service
CD	Commission Decision
CFU	colony forming unit
CV	coefficient of variation
DM	dry matter
EINECS	European Inventory of Existing Chemical Substances
EURL	European Union Reference Laboratory
FCR	feed conversion ratio
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FGE	food group evaluation
IUPAC	International Union of Pure and Applied Chemistry
LOD	limit of detection
LOQ	limit of quantification
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
NOAEL	no observed adverse effect level
SCF	Scientific Committee on Food