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Safety and efficacy of a feed additive consisting of iron chelate of ethylenediamine for all animal species (Zinpro Animal Nutrition (Europe), Inc.)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of iron chelate of ethylenediamine (Iron-EDA-Cl) as a feed additive for all animal species. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) identified several issues related to the data provided concerning the chemical characteristics of the additive. Based on the information provided, the FEEDAP Panel considered unlikely that the additive consists only of iron monochelate of EDA, but of several coexisting (iron) species; therefore, in the absence of adequate experimental data and owing to the uncertainties identified, the Panel cannot conclude on its identity and characterisation. The FEEDAP Panel concludes that the additive is safe for poultry for fattening and reared for laying/breeding, but cannot conclude on the safety for other animal species/categories. The FEEDAP Panel cannot conclude on the safety of the additive for the consumer or the environment. The FEEDAP Panel concludes that handling the additive poses a risk to users by inhalation. The additive should be considered as non-irritant for the skin but irritant for the eyes and skin sensitiser. The FEEDAP Panel cannot conclude on the efficacy of the additive.

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Keywords: nutritional additives, compounds of trace elements, iron chelate of ethylenediamine, Iron-EDA, safety, efficacy

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Table of contents

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 a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Zinpro Animal Nutrition (Europe), Inc.[2] for authorisation of the product iron chelate of ethylenediamine, 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). The particulars and documents in support of the application were considered valid by EFSA as of 7 February 2019.

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 product iron chelate of ethylenediamine, when used under the proposed conditions of use (see Section 3.1.5).

1.2. Additional information

Iron chelate of ethylenediamine (EDA) is intended to be used as a source of iron in all animal species. The additive has not been previously authorised as feed additive in the European Union (EU).

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 chelate of EDA as a feed additive.

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

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the iron chelate of EDA in animal feed. The Executive Summary of the EURL report can be found in Annex A.[4]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of iron chelate of EDA is in line with the principles laid down in Regulation (EC) No 429/2008[5] and the relevant guidance documents: Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008), Guidance for the preparation of dossiers for nutritional additives (EFSA FEEDAP Panel, 2012a), Technical guidance Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012b), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b) and Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017c).

[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 (Europe), Inc.; Akkerdistel 2E - 5831PJ - Boxmeer - The Netherlands.
[3] FEED dossier reference: FAD-2018-0086.
[4] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/jrcsh/files/finirep-fad-2018-0086-feeda.pdf
[5] 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.

3. Assessment

The additive under assessment is iron chelate of EDA,[6] and will be referred from here onwards as Iron-EDA-Cl. It is intended to be used as a nutritional additive (functional group: compounds of trace elements) for all animal species and categories.

3.1. Characterisation

3.1.1. Manufacturing process

[redacted]

3.1.2. Identity of the additive

Five batches of the product were analysed for iron, EDA, moisture and chloride. The average content of iron was about 20.9% (range: 20.6–21.2%), EDA 25.5% (21.9–28.2%), chloride 44.7% (42.61–45.95%) and moisture 0.6% (0.6–0.7%);[8,9] in addition to these data, the applicant provided the content of bound-water which was on average 8.8% (5.7–13.2%).[10]

The applicant provided experimental data to support the amount of chelated and free iron in the additive. Five batches of the additive were analysed; the amount of bound iron averaged 97.8%, with all the analyses showing more than 96.6%.

Based on the available information and knowledge, the applicant made an attempt to provide a chemical description of the additive under assessment. The following characteristics were provided for Iron-EDA-Cl:

- IUPAC Name, Chloro-ethane-(1- ammonium-2-amine)-iron (II) chloride dihydrate.
- The compound is not identified by a Chemical Abstracts Service (CAS) number.
- Molecular weight, 259.34 g/mol.
- Chemical formula, $C_2H_{13}Cl_3N_2O_2Fe$.

The structural formula, as proposed by the applicant (Figure 1), describes the iron ion (Fe^{2+}) as hexa-coordinated by two nitrogen atoms of a single EDA molecule, one of the two being protonated, one water molecule and three chloride ions, resulting in a neutral compound. The theoretical composition, based on the proposed structural formula would be 21.5% iron, 23.6% EDA, 41.0% chloride, 8.78% bound water and 0.6% moisture.

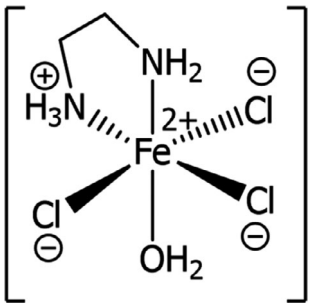

Figure 1: Structural formula of iron chelate of ethylenediamine, as provided by the applicant

[6] Other names used in the dossier: 'Iron chelate of EDA', 'Iron chelate of ethylenediamine', 'Iron-EDA', 'Iron-Ethylenediamine', 'Iron-Ethylenediamine complex', 'FeEDA' and 'Fe-EDA'. Technical Dossier/Supplementary information (May 2019).
[7] Technical dossier/Section II/2.3.
[8] Technical dossier/Section II/Annexes II-28–29.
[9] Technical dossier/Section II/Annexes II-1–5.
[10] Technical Dossier/Supplementary information (September 2020)/Annex: 1. Iron EDA Identity clarification.pdf

The FEEDAP Panel identified the following issues related to the proposed structural formula: i) the protonated nature of one of the nitrogen atoms in the EDA ligand makes the donation of the pair of electrons to iron for the formation of a coordinate bond unlikely, ii) the theoretical composition, calculated from the proposed structural formula, showed deviations for bound water, chloride, iron and EDA, when compared to the analytical data, and iii) the structural formula, as proposed by the applicant, would not match with the IUPAC name, particularly concerning the number and the role of chloride ions (as ligands or counterions). Moreover, the FEEDAP Panel has reservations on the soundness of the proposed IUPAC name.

The FEEDAP Panel further notes that no supporting evidence was provided to substantiate the proposed structural formula, with the exception of infra-red analyses of the compound, without any description of the analytical conditions and a proper interpretation.[11] No evidence was provided to demonstrate that i) the additive is a monochelate of iron with EDA, ii) chloride ions act (at least in part) as ligands rather than as counterions and iii) iron is hexa- rather than tetra-coordinated.

On the other hand, the existence of different iron chelates with EDA, including the mono-, bis- and tris(ethylenediamine)iron(II) complexes has been widely reported in the literature (Bennet et al., 1990) and the occurrence of mono- and bis (EDA)iron(II) complexes was also shown in an *in vitro* dissociation study (see Section 3.2.2.1). [redacted]

Considering all the above, the FEEDAP Panel is therefore unable to confirm the identity of the additive. The remaining analysis provided to support the characterisation of the additive is described in the paragraphs below.

Five batches of the additive were analysed for undesirable substances. Levels of heavy metals (cadmium: 0.23 mg/kg (0.19–0.27 mg/kg), lead: 2.92 mg/kg (2.6–3.2 mg/kg) and mercury: < 0.05 mg/kg), arsenic: < 0.1 mg/kg) and fluorine: < 1.5 mg/kg) were reported.[13] In six batches, the levels of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDDs/Fs) and the sum of PCDD/F and dioxin-like polychlorinated biphenyls (PCBs) were 0.03–0.15 ng WHO-PCDD/F-TEQ/kg and 0.09–0.27 ng WHO-PCDD/F-PCB-TEQ/kg, respectively; the concentration of non-dioxin-like PCBs was 0.03–0.15 ng WHO-PCDD/F-TEQ/kg.[14] The concentrations of the undesirable substances analysed comply with the limits set in Directive 2002/32/EC for compounds of trace elements[15] or, if not mentioned in the Directive, do not represent a concern. The nickel content of the additive (analysis of three batches) showed an average of 0.83 mg/kg (range 0.62–1.19).[16]

Three batches of the additive were analysed for microbial contamination. Counts of Enterobacteriaceae, moulds and yeasts were < 10 colony forming units (CFU)/g and *Salmonella* was not detected in a 25 g sample.[17] Levels of aflatoxin B1 and ochratoxin A analysed in three batches were below the limit of detection (LOD < 0.1 μg/kg).[18]

3.1.3. Physical properties of the additive

The additive is a powder with a bulk density of 980 kg/m^3 (average of three batches).[19] The applicant declared that the product is soluble in water and slightly soluble in methyl alcohol and in ethyl alcohol, while it is practically insoluble in ethyl acetate[20]; however, no supporting data was made available.

[11] The applicant provided the infra-red absorption spectra for three lots of the additive to identify the NH_2 peaks, showing peaks at three wavelengths (1,632.8, 1,633.8 and 1,634.8 cm^{-1}); Technical dossier/Section II/2.2.2.1.
[12] Iron species measured in the dissociation study (see Section 3.2.2.1): Iron species measured in the dissociation study (see Section 3.2.2.1): Fe^{2+}, $[Fe(EDA)]^{2+}$, $[Fe(EDA)_2]^{2+}$, $[Fe(OH)]^{+}$ and $Fe(OH)_2$.
[13] Technical dossier/Section II/Annexes II-10–14.
[14] Technical dossier/Section II/Annexes II-15–19.
[15] 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.
[16] Technical Dossier/Supplementary information (May 2019).
[17] Technical dossier/Section II/Annex II-20.
[18] Technical dossier/Section II/Annexes II-23–25.
[19] Technical dossier/Section II/Table 2.6.
[20] Technical dossier/Section II/Annex II-30.

Dusting potential was analysed by the Stauber–Heubach method in the same three batches as the particle size distribution; four measures were taken on each batch.[21] The results showed a dusting potential ranging from 23.4 to 26.6 g/m^3 air.[21] The same batches were submitted for analysis of the particle size by laser diffraction. The results (v/v) showed an average of 6.91% (range 6.80–7.09), 25.43% (range 25.04–26.18) and 37.62% (range 37.10–38.61) for particles < 10 μm, < 50 μm and < 100 μm, respectively. The applicant provided data on the iron content of the dust measured in the same samples (total of 15 samples subsamples); the average iron content was 217 g Fe/kg dust (range 205–225 mg Fe/kg).[22]

3.1.4. Stability and homogeneity

Although compounds of trace elements (including chelates) stability studies are generally not required, the applicant provided information on the stability of the additive in premixtures and feed for chickens for fattening (mash and pelleted).

The premixture containing the additive was stored for six months, while the mash and pelleted feed for three months (at room temperature). At the end of the experiment, the content of iron in the premixture was 97.6% that initially measured; in the mash and pelleted feed the measured content at six months was equal to the one measured at time zero.

The capacity of the additive to homogeneously distribute in a premixture (vitamin–mineral) and complete feed (mash and pelleted) for chickens for fattening was investigated[23]; the iron content was analysed in ten subsamples each. The coefficient of variation (CV) of the iron concentration in the premixture (mean 66,056 mg/kg) was 4.1%. The CV of the mash feed was up to 3.7%; on the same feed after pelleting (mean iron content: 435 mg/kg) the CV was up to 3.3%.

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 total iron content in complete feed of 500 mg/kg (ovine), 450 mg/kg (bovine and poultry), 250 mg/kg (piglets until 1 week before weaning), 600 mg/kg (pets) and 750 mg/kg (other species).

3.2. Safety

3.2.1. Metabolic studies

No data concerning the metabolic fate of Iron-EDA-Cl have been submitted.

Upon the FEEDAP Panel's request of data on the potential dissociation of the additive in the gastrointestinal tract, the applicant submitted an *in vitro* study of the dissociation of the additive in gastrointestinal and ruminal fluids.[24]

[redacted]

[21] Technical dossier/Section II/Annex II-26.
[22] Technical dossier/Section II/Annex II-26 and Supplementary Information September 2020.
[23] Technical dossier/Section II/Annex II-27.
[24] Technical dossier/Supplementary information April 2020/2. FeEDA dissociation analysis report rev 2 – Confidential.pdf.

However, due to the uncertainties related to the identity of the additive, and the limitations identified in the methodology of the dissociation study, a final conclusion from the study, including an extrapolation to the *in vivo* conditions, could not be drawn.

3.2.1.1. Residue study

In the tolerance study in chickens for fattening (see Section 3.2.3), iron and EDA deposition in liver, kidneys, muscle (breast muscle), bones and skin/fat were measured after 35—37 days administration of the additive at levels corresponding to 40, 350 and 650 mg Fe/kg complete feed; measurements were done in 12 birds per treatment. Iron deposition was compared to that resulting from the administration in the same conditions and levels of iron from iron sulfate. Background level of Fe in the basal diet amounted to 100 mg/kg complete feed.

Iron deposition

Total iron was analysed in tissues after microwave digestion of samples with nitric acid at 230°C using an inductively coupled plasma-mass spectrometry (ICP-MS); the analytical method was validated in-house with a LOD of 0.13 mg/kg and a limit of quantification (LOQ) of 0.38 mg/kg. The results are reported on Table 3. No significant differences in Fe content were found in the tissues sampled among all 7 treatments.

Table 1: Iron deposition (mg/100 g wet tissue) in tissues from chickens for fattening fed different amounts of Iron-EDA-Cl or iron sulfate at 35–37 days

Source	Added Fe (mg/kg)	Total Fe (mg/kg)	Liver	Muscle	Skin/fat	Kidneys	Tibiotarsus
Control	0	100	11.03	0.38	0.48	4.09	29.44
Iron-EDA-Cl	40	140	14.56	0.36	0.51	4.01	29.28
	350	450	14.63	0.36	0.54	4.48	29.73
	650	750	11.39	0.37	0.54	4.14	31.42
Iron sulfate	40	140	17.26	0.34	0.53	4.19	30.05
	350	450	13.45	0.37	0.56	4.16	30.63
	650	750	16.92	0.35	0.58	4.47	32.76

EDA deposition

Total EDA in tissues was analysed after extraction with diluted hydrochloric acid and dissolution of extracts with acetonitrile containing 0.1% formic acid via liquid chromatography–mass spectrometry (LC–MS)[25]; the analytical method was validated in-house with LOD of 5 µg/L, LOQ of 10 µg/L (corresponding to 0.8 mg/kg chicken tissue extract considering a dilution of × 80). The results

[25] Technical Dossier/Supplementary Information May 2020.

provided by the applicant were not coherent with the LOQ described in the validation report[26]; therefore, the applicant was invited to re-submit the complete raw data on EDA residues considering the LOQ of 0.8 mg/kg.

However, the FEEDAP Panel could not assess the new data provided by the applicant as some inconsistencies/limitations were identified (e.g. negative values were reported for a vast number of observations, the LOQ of 0.8 was not applied).[27]

Moreover, no data on residues of iron and EDA in the tissues and products (milk, egg) of other target animal species administered the additive were made available.[28]

3.2.2. Toxicological studies

The applicant provided information supporting the toxicological profile of the additive.

With the exception of genotoxicity studies, for which the item tested was Iron-EDA-Cl, the applicant provided separated data on zinc toxicity and ethylenediamine dihydrochloride (EDA·2HCl) toxicity.

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 approximately 20 mg/kg body weight (bw), is associated with gastrointestinal irritation, while 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).

3.2.2.1. Genotoxicity studies

The dossier includes two genotoxicity studies (bacterial reverse mutation assay and *in vitro* mammalian cell micronucleus test) performed with Iron-EDA-Cl.

- Bacterial reverse gene mutation assay[29]

In order to investigate the potential of Iron-EDA-Cl (batch EPL2078883, Fe 19.96%, purity unknown, see Appendix Nr 4) to induce gene mutations in bacteria, the Ames test was performed according to OECD Test Guideline 471[30] and following Good Laboratory Practice (GLP) in *Salmonella* Typhimurium strains [redacted] In one experiment the increased number of revertant colonies was within the negative historical control range. The Panel concluded that Iron-EDA-Cl did not induce biologically

[26] The applicant indicated directly in the study report an LOQ of 5 mg/kg but the FEEDAP Panel considered valid the LOQ reported in the laboratory's report of the validation of the analytical method (i.e. 0.8 mg/kg). Technical Dossier/Supplementary information (September 2020).
[27] Technical Dossier/Clarification email from the applicant (27.10.2020).
[28] Technical Dossier/Supplementary information (March 2020).
[29] Technical dossier/Section III/Annex III_3_2 Pasteur Instit_OECD471_Ames_FeEDA_Oct 2017.pdf
[30] OECD Guideline Version of 1997. Available online: https://www.oecd.org/chemicalsafety/risk-assessment/1948418.pdf

relevant increase of gene mutations in bacteria under the experimental conditions employed in this study.

- *In vitro* mammalian cell micronucleus test[31]

An *in vitro* micronucleus test was performed according to OECD test guideline TG 487[32] and following GLP to evaluate the potential of Iron-EDA-Cl (batch EPL2078883, Fe 19.96%, purity unknown, see Appendix Nr 5) to induce chromosome damage in TK6 lymphoblastoid human cells in the absence and presence of metabolic activation.

The compound was tested at concentrations ranging from [redacted] No significant increase of micronucleated cells was induced by exposure to Iron-EDA-Cl compared to concurrent vehicle controls. The Panel concluded that the test item did not induce micronuclei in mammalian cells under the experimental conditions employed in this study.

3.2.2.2. Subchronic oral toxicity study in rats

In a non-GLP study,[33] Fischer 344 rats (10 animals/sex per group) were fed EDA·2HCl at 0, 50, 250, 1,000 mg/kg per day (equivalent to 0, 23, 113 and 452 EDA mg/kg bw per day) for 90 days. Investigated toxicity parameters were body weights, food and water consumption, haematology parameters and a limited number of clinical chemistry parameters (glucose, urea nitrogen, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, albumin and creatinine). At necropsy, organ weights were recorded for brain, liver, kidneys, spleen, heart, adrenals and testes. Tissues were collected and subjected to microscopic evaluation.

Marked significant decreases in body weight gain were observed in both sexes, and food consumption in females at 1,000 mg/kg bw per day. A dose related water consumption decrease was observed in females, but given that this effect was minimal (about 1.95 mL water per rat and day) it was considered to be of no toxicological relevance.

In males and females at 1,000 mg/kg bw per day, a significant decrease in absolute and relative liver weights was observed. In addition, males at this level showed a statistically significant decrease in absolute and relative spleen weights. Other statistically significant organ weights changes were reported in both sexes, however, were not considered toxicologically relevant because of the lack of dose response and/or values were similar to one of the two concurrent controls or were considered a result of the marked body weight gain reduction.

A slight decrease in the red blood cell counts and a slight increase in mean corpuscular volume were observed in both sexes at 1,000 mg/kg bw per day. Additionally, in females, a slight decrease in haematocrit and haemoglobin and a slight increase in mean corpuscular haemoglobin were reported. While these changes were dose-related, the small magnitude of these changes is not considered of toxicological relevance or of enough adversity to describe a clinical state of anaemia.

A statistically significant serum glucose level reduction and an increase of alkaline phosphatase activity, AST and ALT activities were reported in both sexes at 1,000 mg/kg bw per day. These findings suggest the probability of an EDA-related effect on the liver of the animals.

A statistically significant lower urine pH was observed in both sexes at 1,000 mg/kg bw per day. This effect may be explained by the known effect of EDA·2HCl as an urine acidifier in human and veterinary medicine. This would also explain the absence of triple phosphate crystals in urine due to an increase of their solubility.

There were no treatment-related gross lesions. The histopathological examination showed an increase in hepatocellular pleomorphism (i.e. cytomegaly, nucleomegaly and multinucleated cells) and occasional mild hepatocellular degeneration at 1,000 mg/kg bw per day.

A no observed adverse effect level (NOAEL) of 250 EDA·2HCl mg/kg bw per day (equivalent to 113 EDA mg/kg bw per day) was identified by the authors, based on reduced body weight gain in both sexes, food and water consumption in females, histopathological effects in liver in both sexes and tracheitis in males observed at 1,000 mg/kg bw per day.

[31] Technical dossier/Section III/Annex III_3_3 Pasteur Instit_OECD487_In vitro Mammalian cell_FeEDA_Oct 2017.pdf
[32] OECD Guideline Version of 2014. Available online: https://www.oecd-ilibrary.org/environment/test-no-487-in-vitro-mammalian-cell-micronucleus-test_9789264224438-en
[33] Technical dossier/Section III/References/11_Hermansky et al_1999.pdf

The FEEDAP Panel notes that this study was not GLP compliant and not performed under the relevant OECD Guideline (Test Guideline 408: Repeated Dose 90-day Oral Toxicity Study in Rodents). Deviations from the regulatory test guideline protocol included the lack of ophthalmological and functional observational battery (FOB) measurements, limited number of haematological and clinical biochemistry parameters measured, and a limited number of organs weighed and histopathologically examined.

3.2.2.3. Chronic oral toxicity studies (including carcinogenicity studies)

In a non-GLP study performed with Fischer 344 rats, EDA·2HCl was fed at 0, 20, 100 or 350 mg/kg bw per day (equivalent to 9, 45 and 158 mg EDA/kg bw per day) for 2 years (Hermansky et al., 1999).[34] Two separate untreated control groups were used. The number of animals of the groups were 100 animals/sex for the low level and the mid-level, and 120 animals/sex for the high level. Interim sacrifices were at 6, 12 and 18 months and the terminal sacrifice was at 24 months. Investigated toxicity parameters were body weights, food and water consumption, a limited number of haematological[35] and clinical biochemistry[36] parameters; a complete urine analysis was conducted in all animals. The evaluation of organ weights was limited to brain, liver, kidneys, spleen, heart, adrenals and testes; the histopathological examination was conducted in a wider range of tissues of all groups.

Most toxic responses were observed at the 12-month sacrifice and thereafter. Reduced body weight gain was observed in males at 350 mg/kg bw per day throughout most of the study and in females at 350 mg/kg bw per day after approximately 18 months. Significant increased mortality was observed in both sexes at 350 mg/kg bw per day and in females at 100 mg/kg bw per day. Most of the deaths occurred after 20 months exposure. The authors indicated that the cause of the decreased survival was unclear but probably ascribable to increased chronic nephropathy.

Erythrocyte counts, haemoglobin concentrations and haematocrit values were generally decreased in males at 350 mg/kg bw per day. Increased urine volume and decreased urine specific gravity was observed in both sexes at 350 mg/kg bw per day in the last half of the study, suggesting a possible alteration in kidney function. These changes reached only significance in males. However, altered urine volume and specific gravity persisted to termination in females only, without significant differences.

Absolute and relative kidney weights were slightly increased in females at 350 mg/kg bw per day during the second half of the study. Absolute and relative liver weights were slightly increased in females (several measurement intervals) and relative liver weights in males at 350 mg/kg bw per day at 24 months. Hepatocellular pleomorphism was observed in both sexes at 350 mg/kg bw per day. In females increase in hepatocellular pleomorphism incidence was reported starting from month 12, while in males at terminal sacrifice. Rhinitis and tracheitis increased in both sexes at 350 mg/kg bw per day.

From this study, a NOAEL of 20 mg EDA·2HCl/kg bw per day (equivalent to 9 EDA mg/kg bw per day) was identified by the authors based on reduced survival in females at 100 mg/kg bw. The authors of the study concluded that EDA·2HCl was not carcinogenic in rats (Hermansky et al., 1999).

The FEEDAP Panel notes that this study was not GLP compliant and not performed under the relevant OECD Guideline (Test Guideline 452: Chronic Toxicity Studies). Deviations from regulatory test guideline protocol included the following: lack of detailed clinical observations, limited number of haematological and clinical biochemistry parameters measured, limited number of organs weighed and histopathologically examined, and lack of ophthalmological measurements and recording of neurological observations.

3.2.2.4. Reproduction toxicity studies

Two studies were assessed

Study 1

In a non-GLP two-generation reproduction study (Yang et al., 1984) Fischer 344 rats were fed a diet containing ethylenediamine dihydrochloride (EDA-2HCl) at levels of 0, 50, 150 and 500 EDA-2HCl mg/kg bw per day (equivalent to 0, 23, 68 and 226 EDA mg/kg bw per day).[37] Parameters examined included indices of fertility, gestation of dams, gestation survival, survival of pups, number of pups born alive, and number of pups weaned per litter. Furthermore, observations were made on mortality, and body weight of the adult rats in F_0 and F_1 generation. Necropsies were performed on F1

[34] Technical dossier/Section III/Annex: 11_Hermansky et al_1999.pdf
[35] Red and white blood cell counts, haemoglobin, mean corpuscular volume, haematocrit, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration.
[36] Glucose, urea nitrogen, total protein, albumin, creatinine, bilirubin (conjugated and total) aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and sorbitol dehydrogenase.
[37] Technical dossier/Section III/5_Yang et al_1984.pdf

weanlings (5 rats/sex per dose, 10 control rats/sex), F1 adults (10 rats/sex per dose, 20 control rats/sex), and F2 weanlings (5 rats/sex per dose, 10 control rats/sex). Organ weights were recorded for the liver, kidneys, spleen, heart, brain, adrenals and testes, for all sacrificed rats. A complete gross necropsy examination was conducted on all sacrificed animals. Tissues (high-dose and control groups; target organs and lesions for all levels) were histologically examined providing an evaluation of the endocrine, cardiovascular, respiratory, gastrointestinal, reproductive, nervous, musculoskeletal and haematopoietic systems.

No treatment-related mortalities were observed. A statistically significant body weight gain reduction was reported in F0 and F1 adult animals at 500 mg/kg bw per day. A minor body weight gain reduction was reported in F0 females at 150 mg/kg bw per day but given the small magnitude of change this finding was not considered of toxicological relevance.

A statistically significant decrease of absolute liver weight was observed in F1 adult males at 500 mg/kg bw per day, and a significant increase of absolute and relative kidney weights was observed in F1 adult females at 150 and 500 mg/kg bw per day. In the absence of histopathological correlates, changes of kidney weight are considered of low toxicological significance.

A statistically significant increased incidence of hepatocellular pleomorphism was observed in F1 adult animals at 500 mg/kg bw per day.

No treatment-related effects on reproduction parameters were reported.

A NOAEL for reproduction of 500 EDA·2HCl mg/kg bw per day (equivalent to 226 EDA mg/kg bw per day) – the highest level tested – was identified by the authors of the study.

A NOAEL for parental toxicity was 150 EDA·2HCl mg/kg bw per day (equivalent to 68 EDA mg/kg bw per day), based on reduced body weight gain and liver histopathological effects in both sexes at 500 mg/kg bw level (Yang et al., 1984).

The Panel notes that this study was not compliant and not performed under the relevant OECD Guideline (Test Guideline 416: Two-Generation Reproduction Toxicity). The main deviations from the current Guidance are i) no pathological investigation was performed in F0 males; ii) no sperm parameters were investigated; however, no reproductive apical effect was observed that could be ascribable to effects on sperms; iii) weights of the following organs were not recorded: uterus, ovaries, prostate, seminal vesicles, pituitary and thyroids. However, it seems that the histopathological examination was performed to evaluate endocrine, cardiovascular, respiratory, gastrointestinal, reproductive, nervous, musculoskeletal and hematopoietic systems.

Study 2

In a non-GLP developmental toxicity study EDA·2HCl was fed to Fischer 344 rats on gestation days (GD) 6 through 15 at levels of 0, 50, 250 and 1,000 EDA·2HCl mg/kg bw per day (equivalent to 0, 23, 113 and 452 EDA mg/kg bw per day) (DePass et al., 1987).[38] Twenty animals per each treatment group were used and 40 served as control timed pregnant. Food consumption and maternal body weight were measured at several intervals during gestation. On GD 21, the foetuses were delivered by caesarean section, and the standard endpoints for teratogenicity were evaluated.

In animals at 1,000 mg/kg bw per day, a statistically significant body weight loss was reported during GD 6–11 and thereafter body weight gain remained significantly reduced until sacrifice when compared to controls. In animals at 250 mg/kg bw per day, body weight gain was significantly reduced during the exposure period (GD 6–15); thereafter, animals gained weight but remained significantly lower in weight than controls until sacrifice. Food consumption was generally significantly lower than controls during the exposure period in animals at 250 and 1,000 mg/kg bw per day.

Toxic effects on fetuses were reduced body weight and crown-rump length, increase of litter incidence with resorptions, skeletal variations and missing or shortened innominate arteries at the highest dose of 1,000 mg/kg bw per day.

To investigate whether the above observed fetal effects could be ascribed to poor nutrition, a follow-up study was conducted in the same laboratory. Two control groups were used: one control group with *ad libitum* access to diet and a pair-feeding control to the EDA group. A third group was fed EDA-2HCl at a level of 1,000 mg/kg bw per day. Results showed that all developmental effects observed in the main study were attributable to EDA-2HCl, and not to food restriction, except for missing innominate arteries.

The authors set a NOAEL for maternal toxicity at 50 EDA-2HCl mg/kg bw per day (equivalent to 23 EDA mg/kg bw per day) based on reduced food intake and body weight gain at 250 mg EDA·2HCl/kg

[38] Technical dossier/Section III/12_DePass at al_1987.docx

bw per day. For developmental toxicity, a NOAEL of 250 EDA·2HCl mg/kg bw per day (equivalent to 113 EDA mg/kg bw per day) was identified, based on foetal weight and crown-rump length reduction, and increased incidences of litter resorptions, skeletal variations and shortened innominate arteries at 1,000 mg EDA·2HCl/kg bw per day. The authors of the study concluded that EDA·2HCl is not teratogenic in Fischer 344 rats. (DePass et al., 1987).

The FEEDAP Panel notes that this study was not GLP compliant and not performed under the relevant OECD Guideline (Test Guideline 41: Prenatal developmental Toxicity). Deviations from regulatory test guideline protocol included the following: no observations for potential clinical signs of toxicity were performed on pregnant animals.

3.2.2.5. Other toxicological studies

The applicant provided a report in which the neurotoxicity of EDA was addressed (WHO, 1999).[39] From the studies described, it was suggested EDA to be a neurotoxic agent, particularly in neonates and in disease states where the blood-brain barrier is incomplete or altered. The potency of this mechanism of action appears to be comparable to that exerted by the gamma-aminobutyric acid (GABA).

3.2.2.6. Conclusions on the toxicological studies

Data from genotoxicity studies performed with the additive did not raise safety concerns.

The toxicological profile of iron is well established and the Panel does not expect any concern from the iron content of Iron-EDA-Cl. Human intake levels of iron below the UL are not associated with any concern for the consumer.

From the toxicological studies submitted with the EDA component of the additive, the Panel identified a lowest NOAEL of 9 mg EDA/kg bw per day based on the rate of mortality observed from a carcinogenicity study conducted in rats fed with EDA-2HCl. However, the Panel identified several limitations in the completeness of the available data (e.g. ophthalmological measurements and functional observational battery are missing). Moreover, the FEEDAP Panel notes that the neurotoxicity of EDA has been suggested. Therefore, owing to the limitations and uncertainties above described, the FEEDAP Panel is not in the position to assess the toxicity of the EDA component of the additive.

3.2.3. Safety for the target species

The applicant provided a tolerance study with Iron-EDA-Cl in chickens for fattening with duration of 35 days.[40,41] This study was designed to support safety for target species and efficacy of the additive, as well as to provide data for the residues' evaluation.

A total of 504 male 1-day-old Cobb 500 was used. The experiment consisted of a randomised complete block design with seven treatments, six pens per treatment and 12 birds per pen.[42] Two basal diets (starter (1–21 days) and grower (22–35 days))[43] based on wheat soya and barley were either not supplemented (control) or supplemented with iron sulfate or Iron-EDA-Cl to reach a total iron level of 140, 450 or 750 mg Fe/kg diet (approx. 0.31, 1 or 1.7 × maximum authorised level), (confirmed by analysis)[44] (Table 2). Diets and water were offered *ad libitum* in crumble (starter) and pelleted (grower) form for 35 days.

Table 2: Experimental design of the tolerance study in chickens for fattening (1–35 days)

Source of added iron	Added iron (mg/kg)[1]	Total intended iron (mg/kg)	Analysed iron (mg/kg)	
			Starter phase	Grower phase
Control	0	100	95	98
Iron-EDA-Cl	40	140	131	137
	350	450	430	421
	650	750	678	751

[39] Technical Dossier/Section III/Reference 20.
[40] Technical dossier/Supplementary information April 2020/FeEDA Signed final T&E report.
[41] Due to the capacity of laboratory to process samples, the necropsies took place over 3 days upon termination of the study (until day 37).
[42] Technical dossier/Section III/III_3_1 DRAFT_BioMedVetFeEDA study report_August 2018.pdf
[43] Technical dossier/Supplementary information April 2020/2. Target Feeds_ZINPRO DIETS AND PREMIXES.pdf
[44] Technical dossier/Supplementary information April 2020/From page 208 to page 227 of the 1. FeEDA Signed final T&E report

Source of added iron	Added iron (mg/kg)[1]	Total intended iron (mg/kg)	Analysed iron (mg/kg)	
			Starter phase	Grower phase
Iron sulfate	40	140	128	133
	350	450	392	449
	650	750	721	749

(1): The basal diet contained 100 mg Fe/kg.

Mortality and health status were monitored daily. Animals were weighed on days 1, 21 and 35 (pen basis); feed intake was registered per pen and feed to gain ratio calculated for the same periods.

Blood samples were obtained from two birds per pen on days 35, 36 and 37 for haematology[45] and blood biochemistry.[46] The same two birds per pen were subject to necropsy and histopathology. From the same animals, samples of liver, kidney, skeletal muscle, skin and fat and tibiotarsus were collected and stored (at nominally –20°C) for iron and ethylenediamine quantification. All the remaining birds were subject to gross pathology examination.

An analysis of variance (ANOVA) was done with the performance data (pen basis) and haematology, blood chemistry and tissue residues (individual basis) and considering the treatment as the effect. Group means were compared with Dunnett's test. The significance level was set at 0.05.

Overall mortality including culling was 4.96% and no significant differences was found between treatments.

No significant differences in performance parameters among treatments were observed, with the exception of a significant increase in feed intake and a reduction in feed to gain ratio in the group receiving 140 mg/kg from iron sulfate when compared to the control group (Table 3).[47] No effects were reported on the haematological and biochemical parameters, and gross pathology.

Table 3: Description of the performance parameters of chickens for fattening from the tolerance study (1–35 days)[(a)]

Source	Added Fe (mg/kg)[1]	Total Fe (mg/kg)	Feed intake (g)	Final weight (g)	Weight gain (g)	Feed/gain ratio	Mortality (including culling)
0	0	100	3,457	2,570	2,525	1.429	11.1
Iron-EDA-Cl	40	140	3,434	2,561	2,517	1.405	6.9
	350	450	3,519	2,599	2,554	1.396	4.2
	650	750	3,442	2,451	2,407	1.435	1.4
Iron sulfate	40	140	3,597*	2,654	2,609	1.381*	1.4
	350	450	3,506	2,563	2,519	1.405	2.8
	650	750	3,497	2,540	2,496	1.418	6.9

(1): The basal diet contained 100 mg Fe/kg.
*: Results are significantly different at $p \leq 0.05$ with respect to the control.
(a): Technical dossier/Supplementary information April 2020/1. FeEDA Signed final T&E report. (appendix 11 end of the Appendix 208 and following).

3.2.3.1. Conclusions on safety for the target species

Based on a tolerance study in chicken for fattening in which birds tolerated up to 1.7 times the maximum authorised level of iron, the Panel concludes that Iron-EDA-Cl is safe for chickens for fattening at the proposed conditions of use. This conclusion is extended/extrapolated to all poultry species for fattening and reared for laying/breeding.

[45] Erythrocytes (RBC), haemoglobin, haematocrit, mean corpuscular volume (MCH), mean corpuscular haemoglobin content (MCHC), mean corpuscular volume (MCV), leukocytes (WBC: eosinophils, lymphocytes, monocytes, heterophils/neutrophils, basophils, platelets).

[46] Sodium, potassium, phosphorus, iron, chloride, calcium, cholesterol, triglycerides, globulin, albumin, total proteins, uric acid, bilirubin, creatinine, glucose, amylase,creatinine kinase (CK),aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), glutamate dehydrogenase (GLDH), lactate dehydrogenase (LDH).

[47] Technical dossier/Supplementary information April 2020/1. FeEDA Signed final T&E report. pdf. (from page 49 VI Tables until page 54).

Considering that the additive under assessment is a chelate compound of a trace element with a xenobiotic substance (EDA), the Panel considers that in order to extrapolate the safety to all animal species, tolerance studies in pigs, cows and salmonids would be required. In the absence of these studies, the Panel is not in a position to conclude on the safety of Iron-EDA-Cl for other species than poultry for fattening/reared for laying/breeding.

3.2.4. Safety for the consumer

Considering (i) the overall uncertainty related to the identity of the additive, (ii) the uncertainty related to the fate of the additive, (iii) the absence of reliable residue data in tissues and products, (iv) the absence of toxicological studies (excluding genotoxicity) with Iron-EDA-Cl and the limitations and uncertainties of the toxicological studies for EDA, the FEEDAP Panel cannot conclude on the safety of the additive for the consumer.

3.2.5. Safety for the user

3.2.5.1. Effects on the respiratory system

No specific inhalation toxicity studies investigating the effects of the additive on the respiratory system were submitted. The submitted safety data sheet provided in the dossier[48] classifies Iron-EDA-Cl as harmful for breathing (P260). Due to the reported dusting potential of the additive (up to 26.6 g/m^3 air; see above Section 3.1.3), an estimation of the iron inhalation exposure was performed.[21]

Taking into consideration, the iron concentration in the dust (average concentration of 217 g Fe/kg dust), a release of 5,772.2 mg Fe/m^3 can be expected when handling the additive.[49] Considering the potential amount of particles of respirable size of the dust, the iron concentration in the respirable dust would be of 1,568.3 mg Fe/m^3.[50] The estimated value is more than three orders of magnitude above the internationally accepted proposed thresholds for iron (1 mg/m^3, set by the American Conference of Governmental Industrial Hygienists as threshold limit value (TLV) (ACGIH, 2008). Consequently, the FEEDAP Panel considers that the additive poses a risk to users upon inhalation.

Uncertainty remains on the effect of the chelate compound in the respiratory system, due to lack of evidence on the fate of the compound in the respiratory tract. However, considering that Iron-EDA-Cl could be dissociated in the lungs, and due to the well-known irritation properties of ethylenediamine (ECHA, 2018a,b), the FEEDAP Panel concludes that the additive poses a risk to users upon inhalation.

Concerning nickel, the additive contains up to 1.19 mg Ni/kg. The dusting potential of the product amounted to an average of 26.6 mg/m^3, corresponding to 0.048 mg Ni/m^3, which is above the occupational exposure limit (OEL) for the inhalable fraction of water-soluble nickel (0.03 mg Ni/m^3; ECHA, 2018a,b). Due to the presence of nickel in the additive, it should be considered as a respiratory sensitiser.

Thus, regarding the effects of the additive on the respiratory system, the FEEDAP Panel considers that handling the additive poses a risk to users by inhalation.

3.2.5.2. Effects on the skin and eyes

The skin irritation potential of Iron-EDA-Cl was tested in a valid study performed according to OECD guideline 404, which showed that it is not a skin irritant.[51]

The eye irritation potential of Iron-EDA-Cl was tested in a valid study performed according to OECD guideline 405, which showed that it is an eye irritant.[52]

Furthermore, the nickel content of the additive is up to 1.19 mg/kg; given its well-known sensitisation potential (European Commission, 2011; ECHA, 2018a,b) and in the absence of skin sensitisation studies the additive should be considered as a skin sensitiser.

[48] Technical dossier/Section II/Annex II-30 Iron-EDA EU SDS_Nov 2018.pdf.
[49] The content of iron in the dust 21.75% (Supplementary Information September 2020 - EFSA FeEDA Supplementary Information Request 20200924.pdf page 4) multiplied by the highest dusting potential (26.6 g/m^3) divided 1000 gives the Fe content in dust (mg Fe/m^3).
[50] Assuming that the dust contains only particles of diameter ($<$ 50 μm), its respirable fraction could be determined as 27.08% (7.09 of 26.18), the iron content in the dust would be 1,566.8 mg/m^3 (27.08 $\times$ 5785.50 mg Fe/m^3 per 100).
[51] Technical dossier/Section III/Annex III_3_4 CERB OECD404_Acute Skin_FeEDA.pdf
[52] Technical dossier/Section III/Annex III_3_5 CERB OECD405_Acute Eye_FeEDA.pdf

3.2.5.3. Conclusions on safety for the user

The FEEDAP Panel concludes that handling the additive poses a risk to users by inhalation. The additive should be considered as non-irritant for the skin but irritant for the eyes and a skin sensitiser.

3.2.6. Safety for the environment

Considering that (i) the data provided in the technical dossier supporting the environmental safety of the additive were not adequate for the assessment (i.e. references to the outcome of environment risk assessment (ERA) on other inorganic and organic iron sources, including chelates with amino acids or glycine from previous FEEDAP Panel opinion (2016), ERA of EDA performed by WHO (1999)) and (ii) the overall uncertainty in the identity of the additive and in its metabolic fate, the FEEDAP Panel cannot conclude on the safety of the additive for the environment.

3.3. Efficacy

For demonstration of the efficacy of nutritional additives, one study in a single animal species or category, including laboratory animals, is generally considered sufficient (EFSA FEEDAP Panel, 2011).

The applicant provided a combined tolerance/residue/efficacy study in chickens for fattening[53] (see Section 3.2.1). The experimental groups are described in Table 1.[54] The study evaluated the efficacy of the feed additive by measuring iron concentration in edible tissues and tibiotarsus in comparison with a control group which contained no added iron and positive control groups which contained added iron as iron sulfate. The results showed that no significant differences were seen in iron deposition among the experimental groups (see Section 3.2.2.2, Table 3). The lack of differences between the control group and the group supplemented with the additive or the inorganic source of iron could be due to the content of iron in the basal diet. Therefore, the study does not provide evidence of the efficacy in iron supplemented groups.

The FEEDAP Panel cannot conclude on the efficacy of the additive for chickens for fattening, and thus for any other 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[55] and Good Manufacturing Practice.

4. Conclusions

In the absence of adequate experimental data and owing to the uncertainties identified in the characterisation of the additive, the Panel cannot conclude on the identity and characterisation.

The FEEDAP Panel concludes that the additive is safe for poultry for fattening and reared for laying/breeding, but cannot conclude on the safety for other animal species/categories.

The FEEDAP Panel cannot conclude on the safety of the additive for the consumer or the environment.

The FEEDAP Panel concludes that handling the additive poses a risk to users by inhalation. The additive should be considered as non-irritant for the skin but irritant for the eyes and skin sensitiser.

The FEEDAP Panel cannot conclude on the efficacy of the additive.

Chronology

Date	Event
17/12/2018	Dossier received by EFSA. Iron chelate of ethylenediamine for all animal species. Submitted by Zinpro Animal Nutrition (Europe), Inc.
17/12/2018	Reception mandate from the European Commission
07/02/2019	Application validated by EFSA – Start of the scientific assessment

[53] Technical dossier/Section IV/Annex IV_4_1 and IV_4_2

[54] Technical dossier/Section IV/IV_4_1 BioMedVet_Tolerance and Efficacy Report in Broilers FeEDA.pdf.

[55] 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.

Date	Event
03/05/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation*
25/06/2019	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 (Addendum) – Scientific assessment suspended. *Issues: safety for target species, safety for consumers, efficacy*
24/04/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
07/05/2019	Comments received from Member States
06/05/2019	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
07/06/2019	Clarification teleconference during Risk Assessment with the applicant according to the "EFSA's Catalogue of support initiatives during the life-cycle of applications for regulated products"
17/07/2020	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation, safety for the consumer*
21/09/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
24/09/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
17/03/2021	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

AAS	atomic absorption spectrometry
ANOVA	analysis of variance
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
bw	body weight
CAS	Chemical Abstracts Service
CFU	colony forming unit
CV	coefficient of variation
ECHA	European Chemicals Agency
EDA	ethylenediamine
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
FOB	functional observational battery
FSA	UK Food Standards Agency
GABA	gamma-aminobutyric acid
GD	gestation days
GLP	Good Laboratory Practice
HILIC	hydrophilic interaction chromatography
ICP-AES	inductively coupled plasma-atomic emission spectrometry
ICP-MS	inductively coupled plasma-mass spectrometry
IUPAC	International Union of Pure and Applied Chemistry
Iron-EDA-Cl	iron chelate of ethylenediamine
LC–MS	liquid chromatography coupled to mass spectrometry
LC–MS/MS	liquid chromatography coupled to mass spectrometry
LOD	limit of detection
LOQ	limit of quantification
NOAEL	no observed adverse effect level

OEL	occupational exposure limit
PCB	polychlorinated biphenyl
PCDD/F	polychlorinated dibenzo-*p*-dioxin and dibenzofuran
TLV	threshold limit value
UL	tolerable upper intake level
WHO	World Health Organization

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for iron chelate of ethylenediamine

In the current application authorisation is sought under Article 4(1) for iron as an *iron chelate of ethylenediamine* preparation 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 the *feed additive* for all categories and species.

Iron chelate of ethylenediamine is a solid preparation for supplementing iron with a minimum content of 20% (w/w) of *iron* and 20% (w/w) of *ethylenediamine (EDA).*

The *feed additive* is intended to be incorporated into premixtures and feedingstuffs according to the maximum levels of *total iron* in the *feedingstuffs* which range from 250 to 750 mg/kg depending on the animal species/category, as established by Regulation (EU) 2017/2330.

For the quantification of *total iron* in the *feed additive*, *premixtures* and *feedingstuffs* the Applicant submitted the internationally recognised ring-trial validated CEN method EN 15621 based on inductively coupled plasma-atomic emission spectrometry ICP-AES after pressure digestion. This method together with the CEN method EN 15510 based on ICP-AES after ashing or wet digestion and the Community method based on atomic absorption spectrometry, which was further ring-trial validated by the UK Food Standards Agency (FSA), were previously evaluated and recommended by the EURL in the frame of the iron group dossier.

In addition, the EURL is aware of two ring-trial validated methods, namely ISO 6869 based on atomic absorption spectrometry (AAS) and EN 17053 based on inductively coupled plasma-mass spectrometry (ICP-MS).

Based on the acceptable method performance characteristics available, the EURL recommends for official control the five ring-trial validated methods: i) EN 15621 and ISO 6869 for the quantification of *total iron* in the *feed additive*, *premixtures* and *feedingstuffs*; ii) EN 15510 for the quantification of *total iron* in premixtures and feedingstuffs; and iii) the Community method (Commission Regulation (EC) No 152/2009 – Annex IV-C) and EN 17053 for the quantification of *total iron* in *feedingstuffs*.

For the quantification of *ethylenediamine* in the *feed additive* the Applicant submitted a single-laboratory validated method based on high performance liquid chromatography coupled to mass spectrometry (LC-MS/MS) detection using a hydrophilic interaction chromatography (HILIC) stationary phase. This method was previously evaluated by the EURL in the frame of the other *ethylenediamine chelate* dossiers for the characterisation of the ligand in the feed additive and it was considered as fit-for-purpose.

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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.