SCIENTIFIC OPINION

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Safety and efficacy of iron oxide black, red and yellow for all animal species

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

Abstract

Iron oxides black, red and yellow are intended to be used as colourings to add and restore colour to feedingstuffs at a recommended concentration between 500 and 1,200 mg/kg. No data on the tolerance of target animals were provided. The iron oxides black, red and yellow are excreted essentially unchanged in the faeces of the target animals. Iron absorption from these water insoluble iron oxides is low. However, no conclusion on the safety of the iron oxides under assessment for the target animals could be made as a sufficient biological and toxicological database, particularly genotoxicity data, was not available. The use of the iron oxides in animal nutrition is unlikely to result in a direct exposure of the consumer and would not influence the iron content of edible tissues and products from animals treated with iron oxides. Consequently, the supplementation of feed for food-producing animals with the iron oxides under assessment would not provide a risk to consumers. Iron oxide black, red and yellow should be considered as irritant to skin and eyes. In the absence of any information, the Panel on Additives and Products or Substances used in Animal Feed FEEDAP cannot conclude on the potential of the additives to be a skin sensitiser. As the inhalation of iron oxides could cause unspecific lung inflammation, inhalation exposure of users should be considered to be a hazard. As there is concern about the possible genotoxicity of iron oxides, any route of exposure should be considered as hazardous. The use of iron oxide black, red and yellow in animal nutrition does not pose a risk to the environment. The iron oxides are effective in colouring feedingstuffs.

Keywords: iron oxide red, hematite, sensory additive, colourant, safety

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Summary

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver an opinion on the safety and efficacy of iron oxide black, red and yellow (E 172) for all animal species. These iron oxides are currently approved for feed and food use.

Iron oxides black, red and yellow are intended to be used as colourings to add and restore colour to feedingstuffs at a recommended concentration between 500 and 1,200 mg/kg.

No data on the tolerance of target animals were provided. The iron oxides black, red and yellow are excreted essentially unchanged in the faeces of the target animals. Iron absorption from these water-insoluble iron oxides is low. However, no conclusion on the safety of the iron oxides under assessment for the target animals could be made as a sufficient biological and toxicological database, particularly genotoxicity data, was not available.

The use of the iron oxides in animal nutrition is unlikely to result in a direct exposure of the consumer and would not influence the iron content of edible tissues and products from animals treated with iron oxides. Consequently, the supplementation of feed for food-producing animals with the iron oxides under assessment would not provide a risk to consumers.

Iron oxide black, red and yellow should be considered as irritant to skin and eyes. In the absence of any information, the FEEDAP Panel cannot conclude on the potential of the additives to be a skin sensitiser. As inhalation of iron oxides could cause unspecific lung inflammation, inhalation exposure of users should be considered to be a hazard. As there is concern about the possible genotoxicity of iron oxides, any route of exposure should be considered as hazardous.

The use of iron oxide black, red and yellow in animal nutrition does not pose a risk to the environment.

The iron oxides are effective in colouring feedingstuffs.

The FEEDAP Panel made a recommendation on the specifications of the iron oxides used as colourings. It also noted that feed compounders should consider the iron content of those colourings that the maximum iron contents set for complete feedingstuffs are respected.

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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 a feed additive shall submit an application in accordance with Article 7. In particular, Article 10(2) of that Regulation also specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, at the latest 1 year before the expiry date of the authorisation given pursuant to Directive 70/524/EEC for additives with a limited authorisation period, and within a maximum of 7 years after the entry into force of this Regulation for additives authorised without a time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from Rockwood Pigments NA, Inc.[2] for re-evaluation of the products iron oxide black, red and yellow, when used as a feed additives for all animals species (category: sensory additives; functional group: (a) colourants: (i) substances that add or restore colour in feedingstuffs).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the applications to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive) and under Article 10(2) (re-evaluation of an authorised feed additive). EFSA received directly from the applicant the technical dossiers in support of these applications. The particulars and documents in support of the applications were considered valid by EFSA as of 14 April 2011.

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 additives comply with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the products iron oxide black, red and yellow, when used under the proposed conditions of use (see Section 3.2.2).

1.2. Additional information

Iron oxide black, red and yellow (E 172) are included in the European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003. They are authorised without a time limit in application of Article 9t (b) of Council Directive 70/524/EEC[3] concerning additives in feedingstuffs (2004/C 50/01) for its use in cats and dogs as colourant additive (colouring agents authorised for colouring foodstuffs by Community rules). The additives are also authorised for all species or categories of animals with the exception of cats and dogs for animal feedingstuffs only in products processed from: (i) waste products of foodstuffs, (ii) other base substances, with the exception of cereals and manioc flour, denaturated by means of these agents or coloured during technical preparation to ensure the necessary identification during manufacture. Under the same Directive, only iron oxide red is authorised for ornamental fish as colourant additive. No maximum levels of iron oxide red in feeds are established in the European Union (EU).

Regulation (EC) No 2112/2003[4] has authorised iron oxide red (Fe_2O_3) as a feed additive belonging to the category 'nutritional additives', functional group 'compounds of trace elements'.

Iron oxide black, red and yellow are approved food colourants in the EU.[5] Maximum permitted levels (MPLs) of iron oxide black, red and yellow are defined in Annex II of Regulation (EC) 1333/2008 on food additives for use in food (authorised at *quantum satis*, except in entire fresh fruit and

[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] On 27/11/2015 EFSA was informed that the applicant Rockwood Pigments NA, Inc. USA, represented in the European Union by Rockwood Italia S.p.A. Divisione Silo, changed to Huntsman Pigments Americas LLC, represented in the EU by Huntsman Pigments S.P.A., Via G. Reiss Romoli 44/12, 10148 (Torino).

[3] List of the authorised additives in feedingstuffs (1) published in application of Article 9t (b) of Council Directive 70/524/EEC concerning additives in feedingstuffs. OJ C 50, 25.2.2004, p. 1.

[4] Commission Regulation (EC) No 2112/2003 of 1 December 2003 correcting Regulation (EC) No 1334/2003 amending the conditions for authorisation of a number of additives in feedingstuffs belonging to the group trace elements. OJ L 317, 2.12.2003, p. 22.

[5] European Parliament and Council Directive 94/36/EC of 30 June 1994 on colours for use in foodstuffs. OJ L 237, 10.9.1994, p. 13.

vegetables (at 6 mg/kg)).[6] The specific purity criteria concerning the use of these additives in foodstuffs are included in Commission Regulation (EU) No 231/2012.[7]

Iron oxides and hydroxide (including iron oxide black, red and yellow) have been evaluated by in the past years by the Scientific Committee for Food (SCF, 1975) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1974, 1975, 1978, 1980, 2000). The International Agency for Research on Cancer (IARC) evaluated hematite and iron oxide in 1972 (WHO-IARC, 1972) and hematite and ferric oxide (Fe_2O_3) in 1987 (WHO-IARC, 1987). The EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS, 2015) adopted a scientific opinion on the re-evaluation of iron oxides and hydroxides (E 172) as food additives.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of technical dossiers[8] in support of the authorisation request for the use of iron oxide black, red and yellow as feed additives. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008[9] and the applicable EFSA guidance documents.

The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA and other expert bodies, 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 active substance in animal feed. The Executive Summary of the EURL report can be found in Annex A.[10]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of iron oxide black, red and yellow is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance for the preparation of dossiers for sensory additives (EFSA FEEDAP Panel, 2012a), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011a), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008), Guidance for the preparation of dossiers for additives already authorised for use in food (EFSA FEEDAP Panel, 2012b), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012c), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012d), and Guidance on the assessment of additives intended to be used in pets and other non food-producing animals (EFSA FEEDAP Panel, 2011b).

3. Assessment

Iron oxide black, red and yellow are applied under the category sensory additives, functional group colourants, subgroup (i) substances that add or restore colour in feedingstuffs for all animal species.

3.1. Characterisation

The specifications for the iron oxides under assessment are the same as those established for the same iron oxides as food additives.[7] The minimum content for total iron in iron oxide black and red is 68% and 60% for iron oxide yellow. The other specifications are < 1% water-soluble matter, arsenic

[6] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16.

[7] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specification for food additives listed in Annex II and III to Regulation 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p. 1.

[8] FEED dossier reference: FAD-2010-0203 (iron oxide black), FAD-2010-0204 (iron oxide red), FAD-2010-0204 (iron oxide yellow).

[9] 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.

[10] The full report is available on the EURL website: http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2010-0202+0203+0204.pdf

< 3 mg/kg, cadmium < 1 mg/kg, chromium < 100 mg/kg, copper < 50 mg/kg, lead < 10 mg/kg, mercury < 1 mg/kg, nickel < 200 mg/kg and zinc < 100 mg/kg.

The applicant stated that the materials/chemicals used in the production of iron oxide black, red and yellow do not contain dioxin, polychlorinated biphenyles or aflatoxins, however, no analytical data were submitted to support this statement, although requested.[11]

3.1.1. Iron oxide black

Iron oxide black (E 172, synonymous CI Pigment Black 11, triiron-tetraoxide, black iron oxide, ferrous ferric oxide, magnetite, Colour Index 77499) is identical to the active substance iron(II,III) oxide (International Union of Pure and Applied Chemistry (IUPAC) name, Chemical Abstracts Service (CAS) number 1317-61-9, molecular formula $FeO{\cdot}Fe_2O_3$, molecular weight 231.55).

Iron oxide black is produced via chemical synthesis using iron oxide yellow (FeO(OH)) or iron oxide red (Fe_2O_3) and/or iron(II) sulfate as starting chemicals which are converted/reacted in the presence of pure oxygen and caustic soda (precipitation process).

Iron oxide black is a black powder with a relative and apparent density of approximately 4.6 kg/L and 0.7 kg/L, respectively. It is insoluble in water and organic solvents, and soluble in mineral acids.

A typical composition of the additive is the following: iron(II,III) oxide 98.4%, sodium sulfate 0.2%, water 0.4%, aluminium oxide 0.5% and magnesium oxide 0.5%.

Five batches of iron oxide black were analysed for the specified contents.[12] They all complied with the specifications (iron oxide black expressed as iron: 68.5–70.9%; water-soluble salts: 0.09–0.13%; arsenic: < 1 mg/kg; cadmium: < 1 mg/kg; chromium: 25–58 mg/kg; copper: 6–31 mg/kg; lead: ≤ 4 mg/kg; mercury: < 0.2 mg/kg; nickel: 57–117 mg/kg; and zinc: 32–65 mg/kg).

3.1.2. Iron oxide red

Iron oxide red (E 172, synonymous di-iron-trioxide, red iron oxide, red ferric oxide, hematite, Pigment Red 101, Colour Index 77491) is identical to the active substance anhydrous iron(III) oxide (IUPAC name, CAS number 1309-37-1, molecular formula Fe_2O_3, molecular weight 159.69).

Iron oxide red is an inorganic synthetic component obtained from iron(II) sulfate by the Penniman–Zoph method.

Iron oxide red is an orange-red to violet-red powder with a relative and apparent density of approximately 5.0 kg/L and 0.7 kg/L, respectively. It is insoluble in water and organic solvents, and soluble in mineral acids.

A typical composition of the additive is the following: Fe_2O_3 99.1%, sodium sulfate 0.3% and water 0.4%.

Five batches of iron oxide red were analysed for the specified contents.[13] They all complied with the specifications (iron oxide red expressed as iron: 68.4–69.7%, water-soluble salts: 0.03–0.07%, arsenic: < 2 mg/kg; cadmium: < 1 mg/kg; chromium: 30–46 mg/kg; copper: 9–38 mg/kg; lead: < 2 mg/kg; mercury: < 0.2 mg/kg; nickel: 102–141 mg/kg; and zinc: 16–55 mg/kg).

3.1.3. Iron oxide yellow

Iron oxide yellow (E 172, synonymous CI Pigment Yellow 42 and 43, iron(III)-α-oxyhydroxide, hydrated iron oxide, ferric oxide hydroxide, goethite, Colour Index 77492), is identical to the active substance iron(III) oxide hydroxide (IUPAC name, CAS number 51274-00-1, molecular formula $FeO(OH){\cdot}H_2O$, molecular weight 88.85 (FeO(OH)).

Iron oxide yellow is an inorganic synthetic component obtained from iron(II) sulfate by the Penniman–Zoph method.

Iron oxide yellow is a pale to dark yellow powder with a relative and apparent density of approximately 4.1 kg/L and 0.4 kg/L, respectively. It is insoluble in water and organic solvents, and soluble in mineral acids.

A typical composition of the additive is the following: iron (III) oxide hydroxide 99%, sodium sulfate 0.3% and water 0.7%.

[11] Technical dossiers FAD-2010-0202, FAD-2010-0203, FAD-2010-0204/Supplementary information/October 2011.
[12] Technical dossier FAD-2010-0203/Supplementary information October 2011/Section II/Annex 1.
[13] Technical dossier FAD-2010-0204/Supplementary information October 2011/Section II/Annex 1.

Five batches of iron oxide yellow were analysed for the specified contents.[14] They all complied with the specifications (iron oxide yellow expressed as iron: 60.5–62.7%; water-soluble salts: 0.16–0.25%; arsenic: < 1 mg/kg; cadmium: < 1 mg/kg; chromium 12–47 mg/kg; copper 7–35 mg/kg; lead: < 3 mg/kg; mercury: < 0.2 mg/kg; nickel: 23–126 mg/kg; zinc: 7–90 mg/kg).

3.2. Physical properties

The original dossier contained data on the particle size obtained by sieve analysis of five batches of each iron oxide. Virtually all particles were below 50 μm.[15] Regarding this outcome, sieve analysis was not considered an appropriate method to determine particle size.

The applicant provided additional data that was also submitted to the EFSA ANS Panel (2015) for the assessment of iron oxides as food additive.[16]

The applicant provided data on particle size distribution by dynamic light scattering and laser diffraction; both methods could not be considered further due to insensitivity to nanoparticles. Further data obtained with transmission electron microscopy (TEM) were provided. TEM identifies two dimensions of particle size as a percentage of particles. The data showed that the particle size distribution varies in relation to the chemistry of the product, so the distributions of primary particle sizes changes from yellow ($FeO(OH)$) to red (Fe_2O_3) to black ($FeO{\cdot}Fe_2O_3$). Iron oxide yellow has the potential for > 50% of the primary particles to be under 100 nm in at least one dimension due to the needle shape that they possess. Iron oxide red showed < 50% primary particles in the nano size range and iron oxide black < 10%.

The dusting potential, determined according to the modified Heubach procedure, was lowest for the iron oxide black (0.3–1.3 g/m^3) followed by iron oxide red (0.8–1.3 g/m^3) and highest for iron oxide yellow (1.4 and 2.1 g/m^3). The inhalable, thoracic and respirable fractions in the dust of the iron oxides measured with a seven cascade impactor were determined in three batches each. The inhalable, thoracic and respirable fractions ranged for the iron oxide black from 177 to 744, 36 to 130 and 15 to 37 mg/m^3, respectively,[17] for the iron oxide red from 426 to 745, 44 to 114 and 15 to 36 mg/m^3, respectively,[17] and for the iron oxide yellow from 829 to 1238, 185 to 279 and 71 to 78 mg/m^3, respectively.[17]

3.2.1. Stability and homogeneity

Stability studies are not required for metal oxides.

As colourants are used to add or restore colour in feedingstuffs and are therefore not intended to have an effect on the animal, homogeneity tests are not required provided that a wide margin of safety to the target animal exists.

3.2.2. Conditions of use

Iron oxides black, red and yellow are intended to be used as colourants in feedingstuffs for all animal species without dose or age restriction. The applicant recommends 500–1,200 mg/kg of the additives in complete (and complementary) feedingstuffs as standard dose.[18]

3.3. Safety

No new data have been provided by the applicant.

The EFSA ANS Panel (2015) adopted a scientific opinion on the re-evaluation of iron oxides and hydroxides (E 172) as food additives. The ANS Panel described the basis of its assessment as follows: 'the Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that became available since then and the data available following

[14] Technical dossier FAD-2010-0202/Supplementary information October 2011/Section II/Annex 1.
[15] Technical dossiers FAD-2010-0202, FAD-2010-0203, FAD-2010-0204/Supplementary information/October 2011/Section II/Answer to question 1.
[16] Technical dossiers FAD-2010-0202, FAD-2010-0203, FAD-2010-0204/Supplementary information/January 2016.
[17] Technical dossiers FAD-2010-0202, FAD-2010-0203, FAD-2010-0204/Supplementary information/October 2011/Section II/Answer to question 2.
[18] Technical dossiers FAD-2010-0202, FAD-2010-0203, FAD-2010-0204/Supplementary information October 2011/Section II/Answer to question 3.

EFSA public calls for data. The Panel noted that some of the original studies, on which previous evaluations were based, were not available for re-evaluation by the Panel'.

The FEEDAP assessment is based on the opinion of EFSA ANS Panel. Extracts of that opinion are provided below.

3.3.1. Toxicology of iron oxides

"In 1974, JECFA allocated a 'Temporary ADI not specified' to iron oxides and hydrated iron oxides due to the lack of information on physiological absorption and iron storage following the use of iron oxides as food pigments. At the 1978 JECFA meeting, this temporary ADI was extended until 1979. In 1980, an ADI of 0–0.5 mg/kg bw/day was established (JECFA, 1980).

The available data indicate that absorption of iron from iron oxides is low. In rats, 0.01–2.3% of the total oral dose of microsized red iron oxide (Fe_2O_3) was absorbed and distributed in different organs or excreted in urine. Low absorption of iron (0.01%) from red iron oxide was observed in humans receiving a diet containing red iron oxide, whereas a higher absorption of yellow iron oxide (1.5–2.4% of the dose) was described in similar populations. In these human studies, the addition of ascorbic acid increased by 5 to 50 times the iron absorption rates from diets containing either red iron oxide (Fe_2O_3) or yellow iron oxide (FeO(OH)). The Panel noted that there are no data regarding the biological fate of microparticles of black iron oxide ($FeO{\cdot}Fe_2O_3$).

Concerning toxicological studies, the Panel noted that there is a lack of information on the presence of nanoparticles in iron oxides used in most of the old studies. Regarding acute toxicity, the available data indicate that iron oxides and hydroxides are of low toxicity in rats and mice.

The subacute oral toxicities of nano red iron oxide (Fe_2O_3-30 nm) and microsized red iron oxide (Fe_2O_3-Bulk) were compared in rats given 0, 30, 300 or 1 000 mg/kg bw/day for 28 days (Kumari et al., 2012). No loss in body weight, no change in feed intake, nor any adverse symptoms and mortality were observed in rats exposed to microsized red iron oxide or to 30 or 300 mg/kg bw/day of red iron oxide nanoparticles. However, rats treated with the high dose of nano red iron oxide (1,000 mg/kg bw/day) showed reduced body weight and feed intake, severe toxic symptoms and several disturbances in biochemical parameters, and adverse histopathological changes in the liver, kidney and spleen. By contrast, microsized red iron oxide did not induce any significant adverse effects in either biochemical parameters or histopathology in rats given the highest dose. This study indicated that the microsized particles i.e. bulk material, are less potent than the nanoparticles in causing toxicity in the exposed animals. From this study, the Panel identified a NOAEL for microsized red iron oxide of 1,000 mg/kg bw per day, the highest dose tested.

No subchronic toxicity studies by oral administration of microsized yellow iron oxide, red iron oxide or black iron oxide were available. A subchronic toxicity of red iron oxide nanoparticles (60–118 nm) was investigated by Yun et al. (2015) in a 13-week oral toxicity study according to the OECD TG 408 (OECD, 1998). Rats received daily doses of 250, 500 or 1 000 mg/kg bw/day for 13 weeks by gavage. Fe_2O_3 nanoparticles had no significant effects on body weight, mean daily food and water consumption when compared to control groups. There were no treatment-related changes in haematological, serum biochemical parameters or histopathological lesions. Some changes observed in organ weights were considered by the authors as not "*toxicologically relevant*". In blood and all tissues tested, including liver, kidney, spleen, lung and brain, the concentration of iron showed no dose-associated response in comparison to the control groups. Iron concentrations in the urine of Fe_2O_3 nanoparticle-treated rats showed no significant differences compared to those of control animals. The authors stated that the subchronic oral dosing with Fe_2O_3 nanoparticles showed no systemic toxicity to rats. The Panel agreed with the conclusion of the authors and identified a NOAEL for nanosized red iron oxide of 1,000 mg/kg bw/day, the highest dose tested in rats receiving Fe_2O_3 nanoparticles by gavage. Owing to the presence of nanoparticles in red iron oxide used as food additive, the Panel considered this study as relevant for the assessment of the safety of red iron oxide.

The Panel noted that using similar range of daily doses, adverse effects were observed in rats subacutely treated (28 days) with red iron oxide nanoparticles whilst no effect was described after a subchronic administration (90 days) of such particles to rats. The Panel considered that this difference could be explained by the use of smaller nanoparticles (30 nm) in the sub-acute study than those used in the subchronic toxicity study (60–118 nm). The former could be more efficiently available to organs and tissues leading to more severe adverse effects.

Red and black iron oxides, both in nano- and microform (7–30 nm and > 100 nm, respectively), were positive in *in vitro* genotoxicity assays in mammalian cells, where induction of DNA strand breaks

and micronuclei was observed. *In vivo* oral administration of both nano- and microsized red iron oxides did not elicit genotoxic effects in rat haemopoietic system, while no data are available for the site-of-contact (gastrointestinal tract). No *in vivo* genotoxicity studies have been performed on black iron oxide and no genotoxicity studies are available for yellow iron oxide. Due to the limitations of the database, and considering the impossibility to read across between iron oxides with different redox state, the Panel considered that the genotoxicity of iron oxides cannot be evaluated based on the available data.

Concerning long-term toxicity and carcinogenicity, no adverse effects were reported in ten dogs maintained from one to nine years on diets containing iron oxide colourant (unspecified compound); the daily consumption was estimated at 428 mg/dog (unpublished study from Carnation Co., 1967, as reported by JECFA, 1983). In a study from Ralston Purina (1967), no adverse effects were reported in cats maintained on diets containing 1,900 mg/kg diet (475 mg/kg bw per day) of iron from iron oxide (equivalent to 0.27% iron oxide) for periods of two to nine years. The IARC Monograph (1987) stated that there was evidence suggesting lack of carcinogenicity of haematite (red iron oxide) and ferric oxide (unspecified compound) to animals, and that there was inadequate evidence of carcinogenicity in humans.

Concerning reproductive and developmental toxicity, no signs of toxicity were observed in an unpublished study (as reported in JECFA, 1983). However, this study was not available and could not be evaluated by the Panel.

In view of assessing the safety of iron oxides and hydroxides, the ANS Panel noted that:

- the particle size distribution of these substances includes particles with one or more dimensions below 100 nm,
- the differences in physical-chemical characteristics of the particulate material (redox states, particle size) between black (which contains iron(II) and iron(III)) and red and yellow (which contain iron(III)) iron oxides could be critical toxicological features,
- the toxicological database on yellow and black iron oxides is very limited,
- genotoxicity data on yellow iron oxide are absent,
- *in vivo* genotoxicity data on black iron oxide are absent,
- *in vivo* genotoxicity data on red iron oxide at the site of contact are absent.

The ANS Panel further considered that read-across from red iron oxide to black iron oxide should not be performed due to differences in their redox states.

In the absence of data on the genotoxicity of yellow iron oxide (FeO(OH)), the Panel noted that read-across from red iron oxide should not be performed due to marked differences in the shape and the size distribution of yellow iron oxide showing a larger fraction of nanosized particles."

The ANS Panel concluded that "an adequate assessment of the safety of E 172 could not be carried out because a sufficient biological and toxicological database was not available."

The FEEDAP Panel endorses the above conclusion of EFSA ANS Panel.

3.3.2. Safety for the target species

No tolerance studies were provided to support the safety for the target species.

Iron compounds with low water solubility, such as iron oxides, are considered to be inefficient dietary sources of iron (National Research Council (NRC), 1998). In studies in piglets, sheep, calves and chickens with different iron sources, Fe_2O_3 showed negligible or no effects on haematological parameters and/or performance of these animal species (Bell and Tucker, 1963; Ammerman et al., 1967; Willingham and Hill, 1970; Ammerman and Miller, 1972; Cornelius and Harmon, 1976). In rats, 0.01–2.3% of the total oral dose of microsized red iron oxide (Fe_2O_3) was absorbed and distributed in different organs or excreted in urine (EFSA ANS Panel, 2015). Suttle (2010) considered Fe_2O_3, used as a colouring agent, as being among the poorest of inorganic iron sources although it is capable of impairing Cu absorption. It should be noted that Fe_2O_3 has been used as an indigestible marker in digestibility studies. It is well-known that dietary factors modify non-haem iron absorption, e.g. ascorbic acid would increase iron absorption while phytate, calcium and polyphenols would decrease (Suttle, 2010). Although the iron status of the organism plays an important role, high iron stores are related to low absorption rates and vice versa.

JECFA mentioned that in its assessment of iron oxides and hydrated iron oxides (1980), the high tolerance of dogs and cats to iron oxide, levels up to 10 g/kg feed, not results in adverse effects.

JECFA further noted that rats consuming more than 50 mg iron oxide/kg body weight (bw) per day for eight generations showed no adverse effects on reproduction.

The EFSA FEEDAP Panel (2016) published an in-depth consideration of the available literature on the maximum tolerated iron concentrations in feed. There were large differences between the animal species. Based on this review, the FEEDAP Panel recommended as maximum safe contents in complete feed 3,000 mg/kg for pigs, 500 mg/kg for ovines, 450 mg/kg for cattle and poultry and 600 mg/kg for cats and dogs. No maximum safe concentration could be identified for horses and fish.

The FEEDAP Panel concluded that (i) a substantial absorption of iron oxides as such is not expected; (ii) iron from the iron oxides black, red and yellow will pass the gastrointestinal tract of target animals essentially unchanged; and (iii) iron from the iron oxides will therefore not measurably contribute to the iron metabolism of target animals.

However, as (i) the application of iron oxides black, red and yellow is for all animals species; (ii) lifetime administration to animals is not excluded; and (iii) a sufficient biological and toxicological database was not available, no conclusion on the safety of the iron oxides under assessment for the target animals could be made.

3.3.3. Safety for the consumer

Although there are no data regarding the biological fate of nano- and microparticles of iron oxides, the FEEDAP Panel considers that the use of iron oxide black, red or yellow in animal nutrition is unlikely to result in a direct exposure of the consumer to these oxides.

Regarding (i) the very low absorption of iron from the iron oxides black, red and yellow by target animals, and (ii) the homoeostatic regulation of iron metabolism in animals, any influence of feeding the iron oxides under assessment on the iron content of edible tissues and products is not expected.

The FEEDAP Panel concludes that the supplementation of feed for food-producing animals with the iron oxides under assessment would likely not constitute a risk to consumers.

3.3.4. Safety for the user

No studies of skin or eye irritancy were provided, but the material safety data sheet (MSDS) noted that irritation might be caused by mechanical action of iron oxide black, red or yellow on skin or eyes.

No studies were provided on skin sensitisation. In the absence of any information, the FEEDAP Panel cannot conclude on the potential of the additives to be skin sensitiser.

Iron oxide black, red and yellow red consist of small particles of respirable size, so there is potential for inhalatory exposure of workers if they are exposed to dust from this material. No inhalation toxicity studies were provided, but the MSDS noted that inhalation can cause coughing, sneezing, respiratory problems and siderosis. Furthermore, IARC-WHO (1972) noted that epidemiological studies of miners potentially exposed to iron ore dust or iron oxide had an increased risk of developing lung cancer, although it was not clear whether the cancer was caused by exposure to radiation or dust in the mines. Studies in mice, hamsters and guinea pigs showed no increased risk of cancer with inhalatory or intratracheal exposure to iron oxides. IARC concluded that Fe_2O_3 is not classifiable as to its carcinogenicity to humans (Group 3, IARC-WHO (1987)).

The toxic effects of iron oxide black nanoparticle (< 50 nm particle size) after a single intratracheal instillation were monitored in adult male Wistar rats. Groups of 30 rats each were administered 0, 1, and 5 mg iron black nanoparticle/kg bw. Lungs and internal organs underwent histopathological examination after 1, 3, 7, 14 and 30 days (six animals per group). There were no pathological changes in examined internal organs, except a very weak pulmonary fibrosis developing by the end of the first month in the treated rats (Szalay et al., 2011).

Iron oxide particles can cross the pulmonary epithelium (Heilig et al., 2006), which indicates a potential for systemic exposure following inhalation. Air concentrations of 16.6 mg magnetite (iron oxide black)/m^3 or more (mass median aerodynamic diameter = 1.3 μm) caused lung inflammation in rats that was typical of non-specific effects of overload with non-toxic material (limit benchmark dose concentration (BMCL) = 3.4 mg/m^3 for increase in lactate dehydrogenase (LDH) in bronchoalveolar lavage fluid, Pauluhn and Wiemann, 2011; Pauluhn, 2012). At all concentrations tested, inhalation of magnetite caused increased number of neutrophils in peripheral blood and in bronchoalveolar fluid, but the biological relevance of this was unclear (Pauluhn, 2012). A level of inhalation exposure without effect has not been identified.

3.3.4.1. Conclusions on safety for the user

Iron oxide black, red and yellow should be considered as irritant to skin and eyes by mechanical action. In the absence of any information, the FEEDAP Panel cannot conclude on the potential of the additives to be a skin sensitiser.

Inhalation of iron oxide black resulted in a lung inflammation in rats, typical of non-specific effects of overload with non-toxic materials. As effects were observed at levels below the concentration of respirable particles in the dust from the iron oxides under assessment, inhalation exposure of users should be considered to be a hazard.

As there is concern about the possible genotoxicity of iron oxides, any route of exposure should be considered as hazardous.

3.3.5. Safety for the environment

Iron oxides and their hydrated forms are ubiquitous in the environment. Any additional input from the nutritional use in food-producing animals is considered negligible. Moreover, iron oxides are insoluble in water, and iron from these compounds has a very low bioavailability. It is unlikely that the use of iron oxide black, red and yellow in animal nutrition would pose a risk for the environment.

3.4. Efficacy

Iron oxides black, red and yellow are intended for use to add or restore colour in feedingstuffs for all animal species. They are approved food colourants.

No further demonstration of efficacy might be necessary (Regulation (EC) No 429/2008) where the function requested for feed is the same as that used in food. However, considering the wide variety of feedingstuffs used in complete and complementary feed, a demonstration of a dose-dependent effect in a typical complementary or complete feedingstuff was requested.

For iron oxide black, the applicant refers to a standard concentration of 500–1,200 mg/kg final product and states that iron oxide black is never used alone as to colour feed but as blends with iron oxide red or iron oxides red and yellow. Pictures of pet food biscuits and animal mash feed (poultry, pig and ruminants) pigmented with blends containing iron oxide black, yellow and red were provided.[18] The data demonstrated that iron oxide black when used in together with iron oxide red has a clearly visible effect on the colour of feedingstuffs at a minimum dose of 700 mg/kg.

For iron oxide red and yellow, the applicant refers to a standard concentration of 500–1,200 mg/kg final product. It provided pictures of pet food biscuits and animal mash feed (poultry, pig and ruminants) pigmented with iron oxide red or yellow and pigmented with blends containing iron oxide black, yellow and red.[18] The data demonstrated that iron oxide red and yellow have a small but visible effect on the colour of feedingstuffs at a minimum dose of 500 mg/kg.

4. Conclusions

The iron oxides black, red and yellow are excreted essentially unchanged in the faeces of the target animals. Iron absorption from these water-insoluble iron oxides is low. However, no conclusion on the safety of the iron oxides under assessment for the target animals could be made, as a sufficient biological and toxicological database, particularly genotoxicity data, was not available.

The use of the iron oxides in animal nutrition is unlikely to result in a direct exposure of the consumer and would not influence the iron content of edible tissues and products from animals treated with iron oxides. Consequently, the supplementation of feed for food-producing animals with the iron oxides under assessment would not provide a risk to consumers.

Iron oxide black, red and yellow should be considered as irritant to skin and eyes. In the absence of any information, the FEEDAP Panel cannot conclude on the potential of the additives to be a skin sensitiser. As inhalation of iron oxides could cause unspecific lung inflammation, inhalation exposure of users should be considered to be a hazard. As there is concern about the possible genotoxicity of iron oxides, any route of exposure should be considered as hazardous.

The use of iron oxide black, red and yellow in animal nutrition does not pose a risk to the environment.

The iron oxides are effective in colouring feedingstuffs.

5. Recommendations

The FEEDAP Panel recommends that the same specifications which are and will be applied to the food grade iron oxides (E 172) should also be applied to the iron oxides used as feed additives.

6. Remark

Maximum contents are set for total iron in complete feed when iron containing compounds are supplemented to feedingstuffs.[19] As the official control does not/cannot differentiate between the sources of iron, and also considering that Fe_2O_3 is listed as a compound of trace elements, iron from iron oxides used to add or restore colour to feedingstuffs will contribute to the total iron in feed. Consequently, the iron content of the ferric oxides should be considered when formulating feed respecting the maximum content of total iron in complete feed. The FEEDAP Panel also notes that iron oxides, in contrast to most of the authorised compounds of iron, would not substantially contribute to meeting the animal requirements for iron.

Documentation provided to EFSA

1) Iron oxide black. September 2010. Submitted by Rockwood Pigments.
2) Iron oxide black. Supplementary information. October 2011. Submitted by Rockwood Pigments.
3) Iron oxide black. Supplementary information. March 2012. Submitted by Rockwood Pigments.
4) Iron oxide black. Supplementary information. January 2016. Submitted by Huntsman Pigments.
5) Iron oxide red. September 2010. Submitted by Rockwood Pigments.
6) Iron oxide red. Supplementary information. October 2011. Submitted by Rockwood Pigments.
7) Iron oxide red. Supplementary information. March 2012. Submitted by Rockwood Pigments.
8) Iron oxide red. Supplementary information. January 2016. Submitted by Huntsman Pigments.
9) Iron oxide yellow. September 2010. Submitted by Rockwood Pigments.
10) Iron oxide yellow. Supplementary information. October 2011. Submitted by Rockwood Pigments.
11) Iron oxide yellow. Supplementary information. March 2012. Submitted by Rockwood Pigments.
12) Iron oxide yellow. Supplementary information. January 2016. Submitted by Huntsman Pigments.
13) Evaluation report of the European Union Reference Laboratory for Feed Additives on the methods(s) of analysis for iron oxides.
14) Comments from Member States.

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[19] OJ L 317, 2.12.2003, p. 22.

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Abbreviations

AAS	atomic absorption spectrometry
ANS	EFSA Panel on Food Additives and Nutrient Sources added to Food
BMCL	limit benchmark dose concentration
bw	body weight
CAS	Chemical Abstracts Service
CEN	European Committee for Standardization
EURL	European Union Reference Laboratory
FAO	Food and Agriculture Organization of the United Nations
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
IARC	International Agency for Research on Cancer
ICP-AES	inductively coupled plasma atomic emission spectroscopy
IUPAC	International Union of Pure and Applied Chemistry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LDH	lactate dehydrogenase
LOQ	limit of quantification
MPL	maximum permitted levels
MSDS	material safety data sheet
NOAEL	no observed adverse effect level
NRC	National Research Council
OECD	Organization for Economic Cooperation and Development
RSD_r	relative standard deviation of *repeatability*
RSD_R	relative standard deviation for *reproducibility*
SCF	Scientific Committee for Food
TEM	transmission electron microscopy
VDLUFA	Association of German Agricultural Analytic and Research Institutes
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 Oxides[10]

Iron oxide (black and *red)* have a minimum content of total iron of 68%, while *Iron oxide (yellow)* has a minimum content of total iron of 60%. All the *Iron oxides* are intended to be incorporated directly in dry or humid *feedingstuffs*, with no recommended minimum or maximum levels.

For the determination of *Iron oxide (yellow, black* and *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. Even though no performance characteristics are provided, the EURL recommends for official control the FAO JECFA monograph based on digestion and iodometric titration for the quantification of the iron in the *feed additive*.

For the quantification of total iron in the *feed additive*, *premixtures* and *feedingstuffs*, the EURL identified two internationally recognised ring-trial validated methods, based on Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES): EN 15510 and CEN/TS 15621, the latter using pressure digestion.

The following performance characteristics were reported for the EN 15510 method, where the total iron content ranged from 293 to 8182 mg/kg:

- a relative standard deviation of *repeatability* (RSD_r) ranging from 2.4 to 4.8%;
- a relative standard deviation for *reproducibility* (RSD_R) ranging from 5.2 to 10.3%; and
- a limit of quantification (LOQ) of 3 mg/kg.

The performance characteristics reported for CEN/TS 15621 method (where the total iron content ranged from 325 to 8550 mg/kg) are: - RSD_r ranging from 1.9 to 5.2%; - RSD_R ranging from 8.1 to 16.4%; and - LOQ = 1 mg/kg *feedingstuffs*.

Furthermore, a Community method is available for the determination of total iron in *feedingstuffs*, with the only performance characteristics reported of LOQ of 20 mg/kg *feedingstuffs*. However, the UK Food Standards Agency recently reported results of a ring-trial based on the above mentioned Community method, and reported precisions (RSD_r and RSD_R) for *feedingstuffs* ranging from 2.3 to 9.5%, for samples containing total iron levels ranging from 196.7 to 339.7 mg/kg *feedingstuffs*. Furthermore, similar results were confirmed by VDLUFA, RSD_r ranging from 0.71 to 5.34% and RSD_R ranging from 5.25 to 9.33% were reported for samples containing total iron levels ranging from 179 to 11700 mg/kg *mineral feedingstuffs* and *premixtures*.

Based on these acceptable method performance characteristics the EURL recommends for official control both CEN methods (EN 15510 or CEN/TS 15621) to determine total iron content by ICP-AES in the *feed additive.* As for the determination of total iron content in *feedingstuffs* and *premixtures*, the EURL recommends for official control the Community method based on Atomic Absorption Spectrometry (AAS) and the above mentioned CEN methods (EN 15510 or CEN/TS 15621).

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.