SCIENTIFIC OPINION

Scientific Opinion on the re-evaluation of iron oxides and hydroxides (E 172) as food additives[1]

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Additives and Nutrient Sources added to Food provides a scientific opinion re-evaluating the safety of iron oxides and hydroxides used as food additives (E 172): yellow iron oxide ($FeO(OH)\cdot H_2O$), red iron oxide (Fe_2O_3) and black iron oxide ($FeO\cdot Fe_2O_3$). Brown Iron Oxide has been included in this assessment for completeness, due to its importance as a commercial blend. The Panel considered that the particle size and particle size distribution should be included in the specifications. In 1980, an ADI of 0-0.5 mg/kg bw/day was established by JECFA. Absorption of iron from iron oxides is low. The acute oral toxicity of iron oxides is greater than 10 g iron oxide/kg bw. From a subacute and a subchronic toxicity study, the Panel identified a NOAEL for red iron oxide of 1 000 mg/kg bw/day, the highest dose tested. Red (Fe_2O_3) and black ($FeO\cdot Fe_2O_3$) iron oxide, both in nano- and micro-form, were positive in *in vitro* genotoxicity assays in mammalian cells. 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 Concerning carcinogenicity and reproductive and developmental toxicity, no signs of toxicity were observed in unpublished studies which were not available and could not be evaluated by the Panel. The 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. Refined exposure estimates show that exposure to E 172 ranged from 0.03 mg/kg bw/day for infants to 3.7 mg/kg bw/day for toddlers at the mean and from 0.1 mg/kg bw/day for infants to 9.5 mg/kg bw/day for toddlers at the 95th percentile for the non-brand-loyal scenario.

KEY WORDS

red iron oxide, black iron oxide, yellow iron oxide-hydroxide, E 172, CI 77492, CI 77491, CI 77499

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SUMMARY

Following a request from the European Commission, the Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to deliver a scientific opinion re-evaluating the safety of iron oxides and hydroxides when used as food additives.

Iron oxides and hydroxides are a group of inorganic pigments collectively allowed for use as food additives (E 172) in the European Union (EU) and previously evaluated by the EU Scientific Committee for Food (SCF) in 1975 and the Joint FAO/WHO Expert Committee of Food Additives (JECFA) in 1974, 1975, 1978, 1980 and 2000 (JECFA, 1974, 1975, 1978, 1980, 2000).

In the European Commission (EC) specifications for iron oxides and hydroxides (E 172) (Commission Regulation (EU) No 231/2012), there are three different oxides: yellow iron oxide ($FeO(OH)\cdot H_2O$), red iron oxide (Fe_2O_3) and black iron oxide ($FeO\cdot Fe_2O_3$). Brown Iron Oxide has been included in this assessment, for completeness due to its importance as a commercial blend: its colour shades are obtained by mixing different amounts of the aforesaid powdered principles. The Panel considered that only material with brown shades obtained by blending of the iron oxides and hydroxides evaluated in this Opinion would be covered by the present assessment.

As these iron oxides and hydroxides have different physical and chemical properties and they can be used separately, the Panel recommended that a clear differentiation (e.g. by adding a, b, c to the E number) be made between the different iron oxides and hydroxides that are currently all included under E 172.

According to the data previously submitted by industry (Rockwood, 2013a), the average particle sizes of iron oxide particles were 1 677, 318 and 957 nm for yellow iron oxide (FeO(OH)), red iron oxide (Fe_2O_3) and black iron oxide (Fe_3O_4), respectively. The Panel noted that the method used by industry for measuring the particle size of iron oxides (Rockwood, 2013a) cannot exclude the presence of particles with one or more dimensions below 100 nm.

More recently, transmission electron microscopy (TEM) analyses were carried out on few E 172 products (Huntsman, 2015). Particle size distributions were found to vary in relation to the chemistry of the product so that the distributions of primary particle sizes changed from FeO(OH) to Fe_2O_3 to Fe_3O_4. In all cases, particles that showed at least one dimension in the nanosize range were detected. The Panel had previously noted that, according to the European Food Safety Authority (EFSA) Guidance document, two different methods should be used to examine the particle size distribution (EFSA Scientific Committee, 2011).

In general, the Panel noted that the manufacturing process of powdered or particulate food additives results in material with a range of sizes. While the mean or median size of the particles is generally significantly greater than 100 nm, a small fraction will always be, and has been, with at least one dimension below 100 nm. The material used for toxicological testing would have contained this nano fraction. The test requirements stipulated in current EFSA guidance documents and EC guidelines for the intended use in the food/feed area apply in principle to unintended nano forms as well as to engineered nano material (ENM).

Therefore, the Panel considered that, in principle, for a specific food additive containing a fraction of particles with at least one dimension below 100 nm, adequately conducted toxicity tests should be able to detect hazards associated with this food additive including its nanoparticulate fraction. The Panel considered that for the re-evaluation of food additives this procedure would be sufficient for evaluating constituent nanoform fraction in accordance with the recommendation of the EFSA Nano Network in 2014.

In 1974, JECFA allocated a 'temporary acceptable daily intake (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 %) was described in similar populations. In these human studies, the addition of ascorbic acid increased by 5–50 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 decrease in body weight, no change in feed intake, nor any adverse symptoms or 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 no-observed-adverse-effect level (NOAEL) for microsized red iron oxide of 1 000 mg/kg bw/day, the highest dose tested. No subacute toxicity studies on yellow ($FeO(OH){\cdot}H_2O$) and black ($FeO{\cdot}Fe_2O_3$) iron oxides were available.

No subchronic toxicity studies by oral administration of microsized yellow iron oxide (FeO(OH)), red iron oxide (Fe_2O_3) or black iron oxide ($FeO{\cdot}Fe_2O_3$) were available. A subchronic toxicity study of various orally administered nanoparticles including red iron oxide (Fe_2O_3, 60-118 nm) was performed by Yun et al. (2015) according to the OECD Test Guideline (TG) 408 (OECD, 1998). Sprague-Dawley rats received daily doses of 250, 500 or 1000 mg/kg bw/day for 13 weeks by gavage. There were no treatment-related changes in haematological, serum biochemical parameters or histopathological lesions. In blood and all tissues tested including liver, kidney, spleen, lung and brain, the concentration of Fe showed no dose-associated response in comparison to the control groups. The authors stated that the subchronic oral dosing with Fe_2O_3 nanoparticles showed no systemic toxicity to rats. The Panel agreed with this statement and identified a NOAEL for nanosized red iron oxide of 1000 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, while 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 subacute 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 (Fe_2O_3) and black ($FeO{\cdot}Fe_2O_3$) 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 fed from 1 to 9 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 Cat Care Center (1968), no adverse effects were reported in cats maintained on diets containing 1 900 mg/kg diet (475 mg/kg bw/day) of iron from iron oxide (equivalent to 0.27 % iron oxide) for periods of 2–9 years. The International Agency for Research on Cancer (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.

The Panel noted that only 10 out of the 49 food categories in which iron oxides and hydroxides (E 172) are authorised could be taken into account in the present exposure estimates and therefore that overall this would result in an underestimation of the actual exposure to iron oxides and hydroxides (E 172) used as food additives in European countries. The Panel noted that due to limited information becoming available on the type of iron oxides and hydroxides (yellow, red or black) used in the authorised food categories, the exposure estimates for E 172 were based on maximum levels/reported use levels irrespectively of the type of iron oxide.

Using the "*maximum level exposure assessment scenario*", mean exposure to iron oxides and hydroxides (E 172) from its use as a food additive ranged from 0.1 mg/kg bw/day for infants to 10.5 mg/kg bw/day for toddlers, while the high exposure using this scenario ranged from 0.2 mg/kg bw/day for infants to 26.9 mg/kg bw/day for toddlers.

Using the *refined brand-loyal assessment exposure scenario*, mean exposure to iron oxides and hydroxides (E 172) from its use as a food additive ranged from 0.1 mg/kg bw/day for infants to 8.9 mg/kg bw/day for toddlers. The high exposure to iron oxides and hydroxides (E 172) using this scenario ranged from 0.2 mg/kg bw/day for infants to 23.1 mg/kg bw/day in toddlers.

Using the *refined non-brand-loyal assessment exposure scenario*, mean exposure to iron oxides and hydroxides (E 172) from its use as a food additive ranged from 0.03 mg/kg bw/day for infants to 3.7 mg/kg bw/day for toddlers. The high exposure to iron oxides and hydroxides (E 172) from its use as food additive using this scenario ranged from 0.1 mg/kg bw/day for infants to 9.5 mg/kg bw/day for toddlers. Overall, the lowest exposure to iron oxides and hydroxides (E 172) was estimated for infants, while the highest exposure was calculated for toddlers, in all scenarios. The food categories that, at the individual level, had the highest contribution to the total individual exposure to iron oxides and hydroxides (E 172) were fine bakery wares.

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

- the particle size distribution of these substances includes particles with one or more dimensions below 100 nm,

- 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 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 size distribution of yellow iron oxide showing a larger fraction of nanosized particles.

Regarding Brown Iron Oxide, the E 172 brown shade is mentioned in Commission Regulation (EU) No 231/2012, although the blend itself is nominally not listed, nor further characterised. The Panel noted that specifications and a reliable toxicological database on yellow, red and black iron oxides are needed in order to assess its safety when used as a food additive.

The 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 Panel noted that for the food additive iron oxides and hydroxides (E 172), the term 'iron oxides' applies sometimes either to iron oxides or iron hydroxides and therefore grouping them together under a single E number is confusing. As these compounds have different physical and chemical properties and they can be used separately, the Panel recommended that a clear differentiation (e.g. by adding a, b, c to the E number) should be made between the different iron oxides and hydroxides that are currently all included under E 172. Furthermore, the Panel noted that concentration data on yellow iron oxide, red iron oxide and black iron oxide alone would be needed for the calculation of exposure estimates for each of the three single iron oxides.

Because of the potential importance of nanoparticles in toxicokinetics and toxicological effects, the Panel considered that the particle size and particle size distribution should be included in the specifications of iron oxides and hydroxides.

The Panel considered that the maximum limits for certain toxic elements (cadmium, arsenic, lead and mercury) present as impurities in the EC specification for iron oxides and hydroxides should be revised in order to ensure that iron oxides and hydroxides (E 172) as food additives will not be a significant source of exposure to these toxic elements in foods. It is also recommended that the limit specified in the EC specifications for chromium should be for the presence of chromium(III) and absence of chromium(VI).

Considering the differences in physical-chemical characteristics of the particulate material (redox state, particle size) between the different iron oxides, the Panel recommended that additional data should be provided on these compounds.

The Panel recommended that the minimum, Tier 1 testing according to the EFSA guidance (2012), should be conducted for the material as marketed as the food additive (E 172):

- red iron oxide: *in vivo* genotoxicity at the site of contact (gastrointestinal tract) and subchronic toxicity,
- yellow iron oxide: a complete set of genotoxicity studies and subchronic toxicity,
- black iron oxide: absorption, distribution, metabolism and excretion (ADME), *in vivo* genotoxicity and subchronic toxicity.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The Regulation (EC) No 1333/2008[4] of the European Parliament and of the Council on food additives requires that food additives are subject to a safety evaluation by the European Food Safety Authority (EFSA) before they are permitted for use in the European Union. In addition, it is foreseen that food additives must be kept under continuous observation and must be re-evaluated by the EFSA.

For this purpose, a programme for the re-evaluation of food additives that were already permitted in the European Union before 20 January 2009 has been set up under Regulation (EU) No 257/2010[5]. This Regulation also foresees that food additives are re-evaluated whenever necessary in the light of changing conditions of use and new scientific information. For efficiency and practical purposes, the re-evaluation should, as far as possible, be conducted by group of food additives according to the main functional class to which they belong.

The order of priorities for the re-evaluation of the currently approved food additives should be set on the basis of the following criteria: the time since the last evaluation of a food additive by the Scientific Committee on Food (SCF) or by EFSA, the availability of new scientific evidence, the extent of use of a food additive in food and the human exposure to the food additive taking also into account the outcome of the Report from the Commission on Dietary Food Additive Intake in the EU[6] of 2001. The report, 'Food additives in Europe 2000[7]' submitted by the Nordic Council of Ministers to the Commission, provides additional information for the prioritisation of additives for re-evaluation. As colours were among the first additives to be evaluated, these food additives should be re-evaluated with the highest priority.

In 2003, the Commission already requested EFSA to start a systematic re-evaluation of authorised food additives. However, as a result of the adoption of Regulation (EU) 257/2010, the 2003 Terms of Reference are replaced by those below.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The Commission asks the European Food Safety Authority to re-evaluate the safety of food additives already permitted in the Union before 2009 and to issue scientific opinions on these additives, taking especially into account the priorities, procedure and deadlines that are enshrined in Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives.

[4] 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.

[5] Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up the programme for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives, OJ L 80, 26.03.2010, p. 19.

[6] Report from the Commission on Dietary Food Additive Intake in the European Union, Brussels, 1 October 2001, COM (2001) 542 final.

[7] Food Additives in Europe 2000, Status of safety assessments of food additives presently permitted in the EU, Nordic Council of Ministers. TemaNord 2002:560.

ASSESSMENT

1. Introduction

The present opinion deals with the re-evaluation of the safety of iron oxides and hydroxides (E 172) when used as food additives.

Iron oxides and hydroxides (E 172) are a group of inorganic pigments collectively authorised as food additives in the EU. They were previously evaluated by the EU Scientific Committee for Food (SCF) in 1975 and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1974, 1975, 1978, 1980 and 2000 (JECFA 1974, 1975, 1978, 1980, 2000).

The Panel was not provided with a newly submitted dossier on iron oxides and hydroxides (E 172) and based its evaluation on previous evaluations, additional literature that has become available since then and the data available following public calls for data[8,9,10]. The Panel noted that not all original studies on which previous evaluations were based were available for re-evaluation by the Panel.

2. Technical data

2.1. Identity of the substance

Iron oxides and hydroxides (E 172) are produced synthetically and consist essentially of anhydrous and/or hydrated iron oxides (Commission Regulation (EU) No 231/2012[11]). Iron oxides and hydroxides also occur naturally, but the natural forms are generally considered unacceptable for use as food colours due to difficulties in ensuring their purity (Emerton, 2008).

Table 1 summarises the chemical information for the food additive E 172, i.e. red and black iron oxides and yellow iron oxide-hydroxide. Information is mainly derived from Commission Regulation (EU) No 231/2012.

Table 1: Identity of iron oxides and hydroxides (E 172) authorised as food additives in the EU (Commission Regulation (EU) No 231/2012)

Chemical name	Chemical formula	Molecular weight (g/mol)	CAS Registry number[a]	Colour Index (CI) number	EINECS (or EC) number	Synonyms[b]
Iron Oxide Yellow: hydrated ferric oxide; hydrated iron(III) oxide	$FeO(OH)\cdot H_2O$	88.85 (FeO(OH))	51274-00-1	77492	257-098-5	CI Pigment Yellow 42 and 43; INS No 172(iii)
Iron Oxide Red: anhydrous ferric oxide; anhydrous iron(III) oxide	Fe_2O_3	159.70	1309-37-1	77491	215-168-2	CI Pigment Red 101 and 102; INS No 172(ii)
Iron Oxide Black: ferroso ferric oxide; iron(II, III) oxide	$FeO\cdot Fe_2O_3$ (Fe_3O_4)	231.55	1317-61-9	77499	235-442-5	CI Pigment Black 11; INS No 172(i)

(a) Not available from the Regulation. CAS Registry numbers obtained from JECFA (2008) and/or REACH classification.
(b) INS identifications obtained from JECFA (2008).

[8] Call for scientific data on food colours to support re-evaluation of all food colours authorised under the EU legislation. Published 8 December 2006. Available online:http://www.efsa.europa.eu/en/dataclosed/call/afc061208.htm

[9] Call for scientific data on iron oxides and hydroxides (E 172) permitted in the EU. Published 16 October 2014. Available online: http://www.efsa.europa.eu/en/dataclosed/call/141016.htm

[10] Call for food additive usage level and/or concentration data in food and beverages intended for human consumption. Available online: Published 27 March 2013. http://www.efsa.europa.eu/en/dataclosed/call/130327

[11] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p. 1-295.

Although the molecular formula of black iron oxide is often written as Fe_3O_4, it is the proportion of ferrous oxide (FeO) which gives the pigment its shade (Rowe, 1984).

Emerton (2008) reported that the chemical composition and hence empirical formulae of the pigments vary according to the method of manufacture. However, the Panel noted that the material of commerce needs to meet the EC specifications.

By mechanically mixing different proportions of the principles yellow iron oxide-hydroxide and red and black iron oxides, a blend characterised by a range of brown hues can be obtained: according to Huntsman (2015), this product is generally marketed as brown iron oxide (or CI Pigment Brown 6) and eventually identified with the same CAS Registry and/or EINECS (or EC) numbers used to identify the constituent principles. The E 172 brown shade is mentioned in Commission Regulation (EU) No 231/2012, although the blend itself is nominally not listed nor further characterised. The Panel noted that a product described as 'iron oxide brown', identified with CAS Registry and EINECS (or EC) numbers 52357-70-7 and 257-870-1, respectively, and possibly obtained as a result of a direct production process, is available from the market, apparently for uses other than food additives. The Panel considered that only material with brown shades obtained by blending of the iron oxides and hydroxides evaluated in this Opinion would be covered by the present assessment.

Table 2 shows the physical properties of the different iron oxides and hydroxides.

Table 2: Physical properties of iron oxides and hydroxides (Rowe, 1984)

Property	Yellow iron oxide-hydroxide	Red iron oxide	Black iron oxide
Particle shape	Acicular	Spheroidal (or acicular if produced from calcination of yellow iron oxide-hydroxide)	Cubical
Density (g/cm^3)	4.1-4.35	5.2-5.4 (or 4.5-5 if produced from calcination of yellow iron oxide-hydroxide)	4.0-4.8
Hardness	Soft	Hard (or softer if produced from calcination of yellow iron oxide-hydroxide)	Medium
Refractive Index	1.9-2.5	2.9-3.2	2.4
Colour (mass tone)	Lemon yellow to dark yellow	Light yellow-red to dark blue-brown	Blue-black

The iron oxides and hydroxides are produced in powdered forms. A critical factor is the particle size and/or distribution, as this has an important bearing on their colour intensity and hue (Emerton, 2008). The particle size is an important property, as changing this, by varying the conditions of manufacture, can produce various shades of the product; this is particularly true with yellow iron oxide and red iron oxide.

All iron oxides and hydroxides are insoluble in water, oil and ethanol, and therefore are used in food as insoluble pigments. They can only be solubilised with concentrated mineral acids, which are not normally associated with food (Emerton, 2008).

Upon request of the Panel for information on the particle size distribution, data were provided by industry (Rockwood, 2013a) regarding the particle size of yellow (FeO(OH)), red (Fe_2O_3) and black (Fe_3O_4) iron oxides when used as food additives. According to the submitted results of batch analysis by dynamic light scattering (DLS) of water-dispersed particles, the average sizes (*Z*-average diameters) of the particles were 1677, 318.1 and 956.9 nm (1.68, 0.32 and 0.96 µm) for yellow iron oxide, red iron oxide and black iron oxide, respectively. Particles falling in the nanosize range (at least one dimension < 0.1 µm) were substantially not detected in the batches analysed. However, the Panel noted that the DLS method is not suitable for detecting particles in the nanosize range.

Data supplied at a later date by Huntsman (formerly Rockwood), obtained by laser diffraction technique (LDT) from the analysis of several batches other than those referred to above, were not consistent with the aforesaid results: for instance, with reference to the principles FeO(OH), Fe_2O_3 and Fe_3O_4 in three selected batches, the average (median) particle sizes were 0.54, 0.59 and 1.57 µm, respectively (Huntsman, 2015). When compared, the features of the two sets of data (distribution patterns, medians, other percentiles, etc.) highlight that an appreciable variability may characterise the size of particles of virtually equivalent chemicals in different batches, a trait that is confirmed when all the data available are considered. Again, nanoparticles appear to be substantially absent in the samples analysed.

With reference to the above results, it must be noted that there are different methods for the determination of particle size. The DLS technique gives an intensity-weighted distribution, where the contribution of each particle in the distribution relates to the intensity of light scattered by the particle itself: as a consequence, coarser particles can deliver signals even orders of magnitude stronger than finer particles do. LDT is a very common measuring technique within the industry: it provides a particle size distribution from a measurement of a liquid-dispersed sample according to a one-dimensional sphere equivalent measuring concept. The results obtained are volume-weighted distributions, i.e. relate to the volume of the particles rather than their number, and exhibit poor sensitivity in the nanosize region: according to Huntsman (2015), 'However, in practice this result represents the likely particle size of the product as supplied to the customer'. Transmission electron microscopy (TEM) is a two-dimensional technique used for the determination of number-based particle size distributions in the nanosize range: as a counting technique, it provides a photograph of the dispersed product and yields a number-weighted distribution, where each particle can be given equal weighting irrespective of its size. This is useful when knowing the absolute number of particles is important or when high resolution (particle by particle analysis) is required. In general, a critical task is separating and counting joined particles into primary particles. Interlaboratory variation may be expected to be high.

TEM analyses were carried out on few E 172 products (Huntsman, 2015). Particle size distributions were found to vary in relation to the chemistry of the product so that the distributions of primary particle sizes changed from FeO(OH) to Fe_2O_3 to Fe_3O_4. In all cases, particles were detected that showed at least one dimension in the nanosize range: FeO(OH) particles were the most irregular (needle-like), whereas Fe_3O_4 particles were potentially the least irregular (cube-like). In conclusion (Huntsman, 2015):

- "yellow food grade iron oxide has potential for >50 number% of the primary particles to be under 100 nm [0.1 µm] in at least one dimension due to the needle shape habit that they possess"
- "red food grade iron oxide [has potential for] <50 number% primary particles in the nanosize range"
- "black food grade iron oxide [has potential for] <10 number% primary particles in the nanosize range".

The Panel had previously noted that, according to the EFSA Guidance document, two different methods should be used to examine the particle size distribution (EFSA Scientific Committee, 2011). In the light of the latest data submitted, the Panel collected evidence that the particle size distributions of iron oxides and hydroxides include particles with one or more dimensions below 100 nm, which if confirmed, may require a specific evaluation.

2.2. Specifications

Specifications have been defined in Commission Regulation (EU) No 231/2012 and by JECFA (2008).

Table 3: Specifications for iron oxides and hydroxides (E 172) according to Commission Regulation (EU) No 231/2012 and by JECFA (2008)

	Commission Regulation (EU) No 231/2012	**JECFA (2008)**
Assay	Yellow: ≥60 % of iron Red: ≥68 % of iron Black: ≥68 % of iron	≥60 % of iron
Description	Powder; yellow, red, brown or black in hue	Yellow, red, brown or black powder
Identification Solubility	Insoluble in water and in organic solvents Soluble in concentrated mineral acids	Insoluble in water and organic solvents; soluble in concentrated mineral acids
Purity		
Loss on drying	–	Iron oxide red: not more than 1.0 % (105°, 4 h)
Water-soluble matter	≤ 1.0 %	≤ 1.0 %
Cadmium	≤ 1 mg/kg	≤ 1 mg/kg
Chromium	≤ 100 mg/kg	–
Copper	≤ 50 mg/kg	–
Arsenic	≤ 3 mg/kg	≤ 3 mg/kg
Lead	≤ 10 mg/kg	≤ 10 mg/kg
Mercury	≤ 1 mg/kg	≤ 1 mg/kg
Nickel	≤ 200 mg/kg	–
Zinc	≤ 100 mg/kg	–

Because of their importance in toxicokinetics and toxicological effects, the Panel considered that particle size and particle size distribution should be included in the specifications of iron oxides and hydroxides (E 172). This should be performed by using appropriate methodologies as presented in the EFSA Guidance document (EFSA Scientific Committee, 2011).

The Panel noted that, for the food additive iron oxides and hydroxides (E 172), the term 'iron oxides' applies sometimes either to iron oxides or iron hydroxides, and therefore grouping them together under a single E number is confusing. As these compounds have different physical and chemical properties and they can be used separately, the Panel recommended that a clear differentiation (e.g. by adding a, b, c to the E number) be made between the different iron oxides and hydroxides that are currently all included under E 172. Accordingly, the toxicological profile and the exposure assessment should be performed for each single food colour.

The Panel noted that iron oxides and hydroxides are not authorised to be used as aluminium lakes for colouring purposes (Commission Regulation (EU) No 231/2012).

The Panel noted that according to the EC specifications for the food additive iron oxides and hydroxides (E 172), the toxic elements cadmium, arsenic, lead and mercury present as impurities are accepted up to a concentration of 1, 3, 10 and 1 mg/kg, respectively. Contamination at these levels could have a significant impact on the exposure to these metals, for which the intake is already close to the health-based guidance values established by EFSA (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2009a, 2009b, 2010, 2012). The Panel considered that the maximum limits for certain toxic elements (cadmium, arsenic, lead and mercury) present as impurities in the EC specification for iron oxides and hydroxides (E 172) should be revised in order to ensure that iron oxides and hydroxides (E 172) as food additives will not be a significant source of exposure to these toxic elements in foods. The Panel noted that the limit specified in the EC specifications for chromium should be for the presence of chromium(III) and the absence of chromium(VI). Furthermore, nickel is permitted up to a concentration of 200 mg/kg in the food additives iron oxides and hydroxides (E 172),

which could markedly increase the dietary exposure and decrease the already low margin of exposure (MOE) for nickel (EFSA, 2015).

2.3. Manufacturing process

Food-grade iron oxides and hydroxides are produced synthetically. Iron oxides for use in foods are distinguished from technical grades by their low level of contamination with other metals. The low level of contamination of food-grade iron oxides is achieved by carefully selecting the source of the iron and by the extent of the chemical purification during the manufacturing process (JECFA, 2008).

Iron oxides and hydroxides are produced in powdered forms (Commission Regulation (EU) No 231/2012). The colour shade in iron oxides and hydroxides depends on the manufacturing process and particle size distribution, with more intense and greater tinctorial strength for smaller particle sizes (Emerton, 2008). The optical properties of the yellow one, that is needle-shaped, depend not only on the particle size but also on the length-to-width ratio (Cornell and Schwertmann, 2003).

There are several processes for the production of high-quality iron oxide and hydroxide pigments with controlled mean particle size, particle size distribution, particle shape, etc. (solid state reactions for red, black and brown; precipitation for yellow, red and black and a Laux process involving the reduction of nitrobenzene for black, yellow and red). In principle, iron oxides can be prepared from aqueous solutions of iron salts through precipitation, the most suitable method for producing pigments with a pure and bright hue (Cornell and Schwertmann, 2003).

Yellow iron oxides (FeO(OH)) are produced by using an alkali to precipitate hydrated ferric oxide from a ferrous salt, which is then followed by oxidation. This process produces a pigment with shades ranging from lemon-yellow to deep yellow (Emerton, 2008).

Red iron oxides (Fe_2O_3) are produced by calcination (at 700-800 °C) of yellow iron oxides which are manufactured as described above (Emerton, 2008). Alternatively, red iron oxides can also be manufactured by calcination or thermal decomposition of ferrous sulphate heptahydrate. In this process, ferrous sulphate heptahydrate is dehydrated to the monohydrate and then roasted at a temperature higher than 480 °C in rotary kilns or reverbatory furnaces. The final colour produced can be controlled to some extent by varying the temperature, pressure and time of calcination. Relatively short calcination durations (7-8 hours) and lower temperatures produce lighter shade pigments, whereas longer calcination durations (11-12 hours) and higher temperatures produce deeper blue/red shades (Rowe, 1984).

Black iron oxides ($FeO{\cdot}Fe_2O_3$) are produced by controlled air oxidation of ferrous hydroxide at approximately 200 °C until the required degree of oxidation is reached and precipitation of magnetite of iron oxide ($FeO{\cdot}Fe_2O_3$) occurs. Hydrated ferrous sulphate is treated with sodium hydroxide and oxygen to form a mixture of ferrous and ferric oxides (Emerton, 2008).

Brown Iron Oxide is manufactured through a blending process of yellow, red and black iron oxides in different proportions to adjust the colour shade, followed by a soft milling process for homogenisation. It has a specific hue depending on the mixture ratio of the three components (Huntsman, 2015).

According to industry, yellow iron oxide and red iron oxide are produced via chemical synthesis using the Penniman–Zoph and precipitation processes. In the first stage, nuclei are prepared by precipitating iron(II)sulphate with sodium hydroxide solution in the presence of pure oxygen. The nuclei suspension is treated with iron powder resulting in a growth reaction of the iron oxide onto the nuclei (Penniman–Zoph process). In the following step, a sieving and magnetic separation is carried out followed by two steps of filtration and washing before the drying step, followed by milling (Rockwood, 2013b, 2013c, Huntsman, 2015). While black iron oxide is produced via chemical synthesis using a precipitation process (Rockwood, 2013d), yellow iron oxide or red iron oxide react with iron(II)sulphate in the presence of pure oxygen and caustic soda (precipitation process) to produce black iron oxide. In all cases, the reaction end point is determined by the colour shade of the as-prepared iron oxides. By

neutralisation of the acidic dispersion, the reaction is stopped. The iron oxides are isolated by filtration and washed with hot deionised water to remove residual salts (by-product of the reaction is sodium sulphate). The pigment paste is dried and ground to obtain a homogeneous particle size distribution.

2.4. Methods of analysis in food

There are different methods available for quantifying iron through different analytical techniques as spectrophotometry, atomic absorption spectrometry (AAS), inductively coupled plasma-atomic emission spectroscopy (ICP-AES), inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma-mass spectrometry (ICP-MS) among others (Scotter, 2011; 2015).

According to JECFA specifications for iron oxides, the proposed method of assay is based on a titrimetric procedure, with a iodometric titration after treatment of the sample with hydrochloric acid and hydrogen peroxide solutions (JECFA, 2008).

The analytical methods for foods found in the literature are used for the determination of total iron or iron species but these are not specific for the analysis of iron oxides and hydroxides. However, there are some methods that have been applied for the determination of iron oxide content in other substrates.

The technique of diffuse reflectance (DR) spectroscopy is useful to identify and characterise different types of iron oxides (Torrent and Barrón, 2002). This technique is based on the different DR spectra, due to the different colour of iron oxides, oxihydroxides and hydroxides. The powdered sample is used directly for the analysis without further processing, but this technique has been tested in mineral samples only.

Tokalioğlu and Gürbüz (2010) have developed a solid phase extraction method for the determination of copper and iron in various food samples by flame AAS, using a previous digestion with nitric acid and hydrogen peroxide. This technique has the advantage of eliminating the interference of matrices in the analysis with preconcentration steps.

Another rapid spectrophotometric method developed by Kosse et al. (2001), for determination of iron concentration in foods, is suitable for monitoring of Fe concentration by measuring the absorbance at 535 nm, and has been validated against the standard method AOAC 14.013.

Ion chromatography coupled with UV–Vis detection is also an appropriate sensitive technique for the simultaneous analysis of iron together with other minerals in food samples. The method detects the formation of mineral complexes with 4-(2-pyridylazo)resorcinol by UV–Vis absorption at 500 nm. The detection limit makes it an alternative to AAS and, in several applications, also an alternative to ICP-MS techniques (Fredrickson et al., 2002).

Inductively coupled plasma optical-emission spectrometry (ICP-OES) is a method that has also been used for the quantification of iron and other minerals content in food (Akpinar et al., 2010).

A spectrophotometric method can also be used for the determination of iron and other elements in foods such as grains, milk and tea using a bis-azo-dye, 2,6-bis(1-hydroxy-2-naphthyl azo)pyridine, after Sharma and Singh (2009).

An improved version of the AOAC Official Method 984.27 for the determination of iron, among other nutritional elements, in fortified food products, including infant formula, by ICP-AES after microwave digestion has been developed through a single laboratory validation and a ring trial in experienced food industry laboratories. The validation of the method was performed to characterise the selectivity, sensitivity, linearity, accuracy, precision, recovery, ruggedness and uncertainty (Poitevin et al., 2009).

Energy-dispersive X-ray fluorescence spectrometry (EDXRF) is a suitable technique for the measurement of iron (Fe) and zinc (Zn) concentration in food matrices such as whole grain, rice and pearl millet (Paltridge, 2012).

Another recent method for the simultaneous determination of iron and other elements in foodstuff is by ICP-MS, after closed-vessel microwave digestion (Chevalier et al., 2015). This method is useful for routine determination of iron and other minerals in foodstuff, with acceptable analytical performance.

The Panel noted that the methods used for the analysis of iron in food do not differentiate between the different chemical forms of iron.

2.5. Reaction and fate in food

Iron oxides and hydroxides are very stable in various light, pH, heat and oxidation conditions of relevance to food (Emerton, 2008).

According to industry (Rockwood, 2013b, 2013c, 2013d), yellow iron oxide (FeO(OH)), red iron oxide (Fe_2O_3) and black iron oxide ($FeO \cdot Fe_2O_3$) are considered to be stable under normal conditions. Yellow iron oxide is stable up to 80 °C; above this temperature, dehydration to red iron oxide is possible. Red iron oxide is stable up to 1 000 °C. Black iron oxide is also stable up to 80 °C; above this temperature, it may be partially or completely oxidised to red iron oxide. Yellow, red and black iron oxides are stable at slightly acid, neutral and basic pH; they are not sensitive to moisture and are not hygroscopic. Yellow and red iron oxides are not subject to further oxidation reactions, being stable in oxygen-containing atmospheres. However, black iron oxide may be subjected to further oxidation reactions; it is stable in oxygen-containing atmospheres up to a temperature of approximately 80 °C.

2.6. Case of need and proposed uses

Maximum levels of iron oxides and hydroxides (E 172) have been defined in Annex II to Regulation (EC) No 1333/2008[12] on food additives, as amended. These levels are referred by the Panel as maximum permitted levels (MPLs) in this document.

Currently, iron oxides and hydroxides (E 172) are authorised food additives in the EU and permitted to be used in foodstuffs at *quantum satis* (QS), except in entire fresh fruit and vegetables (at 6 mg/kg). Iron oxides and hydroxides (E 172) are included in the Group II of food colours authorised at QS.

Table 4 summarises foods that are permitted to contain iron oxides and hydroxides (E 172) and the corresponding MPLs as set by Annex II to Regulation (EC) No 1333/2008.

[12] 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.

Table 4: MPLs of iron oxides and hydroxides (E 172) in foods according to Annex II to Regulation (EC) No 1333/2008

FCS category number [(a)]	FCS food category	E-number/ Group	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
01.4	Flavoured fermented milk products including heat-treated products	Group II		*Quantum satis*
01.5	Dehydrated milk as defined by Directive 2001/114/EC	Group II	Except unflavoured products	*Quantum satis*
01.6.3	Other creams	Group II	Only flavoured creams	*Quantum satis*
01.7.1	Unripened cheese excluding products falling in category 16	Group II	Only flavoured unripened cheese	*Quantum satis*
01.7.3	Edible cheese rind	Group II		*Quantum satis*
01.7.4	Whey cheese	Group II		*Quantum satis*
01.7.5	Processed cheese	Group II	Only flavoured processed cheese	*Quantum satis*
01.7.6	Cheese products (excluding products falling in category 16	Group II	Only flavoured unripened products	*Quantum satis*
01.8	Dairy analogues, including beverage whiteners	Group II		*Quantum satis*
03	Edible ices	Group II		*Quantum satis*
04.1.1	Entire fresh fruit and vegetables	E 172	Only as a contrast enhancer for marking citrus fruit, melons and pomegranates in order to: repeat all or some of the mandatory information particulars required by the Union legislation and/or national law, and/or provide on a voluntary basis brand name, production method, PLU-code, QR-code and/or barcode	6
04.2.4.1	Fruit and vegetable preparations excluding compote	Group II	Only mostarda di frutta	*Quantum satis*
04.2.4.1	Fruit and vegetable preparations excluding compote	E 172	Only seaweed based fish roe analogues	*Quantum satis*
04.2.5.3	Other similar fruit or vegetable spreads	Group II	Except *crème de pruneaux*	*Quantum satis*
05.2	Other confectionery including breath freshening microsweets	Group II		*Quantum satis*
05.3	Chewing gum	Group II		*Quantum satis*
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	Group II		*Quantum satis*
06.3	Breakfast cereals	Group II	Only breakfast cereals other than extruded, puffed and/or fruit-flavoured breakfast cereals	*Quantum satis*
06.5	Noodles	Group II		*Quantum satis*
06.6	Batters	Group II		*Quantum satis*
06.7	Pre-cooked or processed cereals	Group II		*Quantum satis*
07.2	Fine bakery wares	Group II		*Quantum satis*
08.3.3	Casings and coatings and decorations for meat	Group II	Except edible external coating of *pasturmas*	*Quantum satis*

FCS category number [(a)]	FCS food category	E-number/ Group	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
09.2	Processed fish and fishery products including molluscs and crustaceans	Group II	Only surimi and similar products and salmon substitute	*Quantum satis*
09.2	Processed fish and fishery products including molluscs and crustaceans	E 172	Only fish paste and crustacean paste	*Quantum satis*
09.2	Processed fish and fishery products including molluscs and crustaceans	E 172	Only smoked fish	*Quantum satis*
09.3	Fish roe	Group II	Except sturgeons' eggs (Caviar)	*Quantum satis*
12.2.2	Seasonings and condiments	Group II	Only seasonings, for example curry powder, tandoori	*Quantum satis*
12.4	Mustard	Group II		*Quantum satis*
12.5	Soups and broths	Group II		*Quantum satis*
12.6	Sauces	Group II	Excluding tomato-based sauces	*Quantum satis*
12.7	Salads and savoury-based sandwich spreads	Group II		*Quantum satis*
12.9	Protein products, excluding products covered in category 1.8	Group II		*Quantum satis*
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	Group II		*Quantum satis*
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)	Group II		*Quantum satis*
13.4	Foods suitable for people intolerant to gluten as defined by Regulation (EC) No 41/2009	Group II		*Quantum satis*
14.1.4	Flavoured drinks	Group II	Excluding chocolate milk and malt products	*Quantum satis*
14.2.3	Cider and perry	Group II	Excluding *cidre bouché*	*Quantum satis*
14.2.4	Fruit wine and made wine	Group II	Excluding *wino owocowe markowe*	*Quantum satis*
14.2.5	Mead	Group II		*Quantum satis*
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008	Group II	Except: spirit drinks as defined in Article 5(1) and sales denominations listed in Annex II, paragraphs 1–14 of Regulation (EC) No 110/2008 and spirits (preceded by the name of the fruit) obtained by maceration and distillation, Geist (with the name of the fruit or the raw material used), London Gin, Sambuca, Maraschino, Marrasquino or Maraskino and Mistrà	*Quantum satis*
14.2.7.3	Aromatised wine-product cocktails	Group II		*Quantum satis*

FCS category number [(a)]	FCS food category	E-number/ Group	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15 % alcohol	Group II		*Quantum satis*
15.1	Potato-, cereal-, flour- or starch-based snacks	Group II		*Quantum satis*
15.2	Processed nuts	Group II		*Quantum satis*
16	Desserts excluding products covered in categories 1, 3 and 4	Group II		*Quantum satis*
17.1	Food supplements supplied in a solid form including capsules and tablets and similar forms, excluding chewable forms	Group II		*Quantum satis*
17.2	Food supplements supplied in a liquid form	Group II		*Quantum satis*
17.3	Food supplements supplied in a syrup-type or chewable form	Group II		*Quantum satis*

(a): FCS, Food Categorisation System (food nomenclature) presented in Annex II to Regulation (EC) No 1333/2008.

2.7. Reported use levels or data on analytical levels of E 172 in food

Most food additives in the EU are authorised at a specific MPL. However, a food additive may be used at a lower level than the MPL. For those food additives for which no MPL is set and which are authorised at QS, information on actual use levels is required for performing an exposure assessment.

In the framework of Regulation (EC) No 1333/2008 on food additives and of Commission Regulation (EU) No 257/2010[13] regarding the re-evaluation of approved food additives, EFSA issued in 2006 a public call[14] for scientific data on food colours, including iron oxides and hydroxides (E 172), to support the re-evaluation of all food colours authorised under the EU legislation. Among other information, the former EFSA Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) was seeking data on present use and use patterns (i.e. which food categories and subcategories, proportion of food within categories/subcategories in which it is used, actual use levels (typical and maximum use levels)), especially for those uses which are limited only by QS. In response to this public call, limited usage data on iron oxides and hydroxides (E 172) were submitted to EFSA by the Confederation of the Food and Drink Industries of the EU (CIAA, currently FoodDrinkEurope) (CIAA, 2009).

In addition, a public call[15] for food additive usage levels and/or concentration data in food and beverages intended for human consumption was launched in March 2013, with a deadline in November 2013. Data on iron oxides and hydroxides (E 172), including present use and use patterns (i.e. which food categories and subcategories contain the additive, the proportion of foods within categories/subcategories in which it is used, and actual use levels (typical and maximum)), were requested from relevant stakeholders. European food manufacturers, national food authorities, research institutions, academics, food business operators and any other interested stakeholders were invited to submit usage and/or concentration data on iron oxides and hydroxides (E 172) in foods. The data submission to EFSA followed the requirements of the EFSA guidance on standard sample description for food and feed (EFSA, 2010).

[14] Call for scientific data on food colours to support re-evaluation of all food colours authorised under the EU legislation. Published 8 December 2006. Available online: http://www.efsa.europa.eu/en/dataclosed/call/afc061208.htm

[15] Call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Published 27 March 2013. Available online: http://www.efsa.europa.eu/en/dataclosed/call/130327.htm

In response to this public call, updated information on the actual use levels of iron oxides and hydroxides (E 172) in foods was made available to EFSA by industry.

No analytical data on the concentration of iron oxides and hydroxides (E 172) in foods were made available by the Member States.

2.7.1. Summarised data on reported use levels of E 172 in foods provided by industry

Industry provided EFSA with data on use levels (n=29) of iron oxides and hydroxides (E 172) in foods for 11 out of the 49 food categories in which iron oxides and hydroxides (E 172) are authorised.

Updated information on the actual use levels of iron oxides and hydroxides (E 172) in foods was made available to EFSA by FoodDrinkEurope (FDE), the International Chewing Gum Association (ICGA), the Association of the European Self-Medication Industry (AESGP) and Capsugel for the following food categories of finished products: edible ices (FCS 03), confectionery (FCS 05.2, 05.3, 05.4), fine bakery wares (FCS 07.2), casings and coatings and decorations for meat (08.3.3), flavoured drinks (FCS 14.1.4), snacks (FCS 15.1), desserts (FCS 16) and food supplements (FCS 17). Most of the use levels reported related to the use of E 172 in food supplements (in particular, FCS 17.1).

Upon request of EFSA, limited information became available by the data providers on the type of iron oxides and hydroxides (yellow, red or black) used in some of the above-mentioned food categories: chewing gum (red and yellow), food supplements (mostly red and yellow), sugar and confectionery (mixtures of red, yellow or black).

For flavoured drinks, only one use level was reported for a niche product (a multivitamin soft drink with sweetener). This level was nevertheless used in the exposure calculations, as no other value was available.

Appendix A provides data on the use levels of iron oxides and hydroxides (E 172) in foods as reported by industry.

The Panel noted that for the most consumed foods, use levels were reported (flavoured drinks and fine bakery wares); however, for many food categories in which the use of iron oxides and hydroxides (E 172) is authorised, no use levels were received by EFSA. Provided that E 172 is indeed used in these foods, some of these food groups i.e. dairy products (FCS 01.4 mainly), breakfast cereals (FCS 06.3), soups and broths (FCS 12.5) and alcoholic beverages (FCS 14.2.3, 14.2.6) could also be important contributors.

The Panel noted that, according to the Mintel GNDP database[16], foods belonging to some of the food categories mentioned above are nevertheless reported to contain iron oxides and hydroxides (E 172): dairy products (e.g. yoghurts with cocoa dragées, cheeses), fish products (smoked fish), protein products (meat substitutes like soya sausages), few sauces and seasonings, savoury spreads and very few wine-based drinks. On the contrary, according to the information available in this database, other foods in which the use of iron oxides and hydroxides (E 172) is authorised were not found to contain E 172: soups, breakfast cereals, some alcoholic drinks (cider, spirits).

2.8. Information on existing authorisations and evaluations

Iron oxides and hydroxides (E 172) are authorised food additives in the EU in accordance with Annex II to Regulation (EC) No 1333/2008 on food additives. Specific purity criteria on iron oxides and hydroxides (E 172) have been defined in the Commission Regulation (EU) No 231/2012.

[16] Mintel Global New Products Database (http://www.mintel.com/global-new-products-database). Accessed on 17/07/2015.

Iron oxides and hydroxides were previously evaluated by the SCF in 1975 and JECFA in 1974, 1975, 1978, 1980 and 2000 (JECFA 1974, 1975, 1978, 1980, 2000). In 1975, the SCF established an Acceptable Daily Intake (ADI) of 'no upper limit specified' in consideration of information showing that only 1 % of the iron oxides and hydrated iron oxides were likely to become solubilised in the human gastrointestinal tract, which would not contribute significantly to the total dietary intake of iron.

In 1974, JECFA established a 'Temporary ADI not specified' for 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).

In the USA, the colour 'synthetic iron oxide' is defined as consisting 'of any one or any combination of synthetically prepared iron oxides, including the hydrated forms. It is free from admixture with other substances' (FDA, 2015; 21CFR73.200). Its use is restricted to 'the coloring of sausage casings intended for human consumption in an amount not exceeding 0.10 per cent by weight of the finished food', and its only other use is to colour cat and dog food (FDA, 2015; 21CFR73.200).

In Australia and New Zealand, iron oxides (INS 172) are permitted in Schedule 3 which refers to colours permitted in accordance with good manufacturing practices (GMP) in processed foods specified in Schedule 1. Schedule 1 gives general provisions for the use of food additives, lists which food additives are permitted in specific foods, the maximum permitted levels and the International Numbering System (INS) numbers (FSANZ, accessed via internet 2015).

In 1987, the International Agency for Research on Cancer (IARC) Monograph reported that for haematite (mineral iron oxide red) and ferric oxide (iron oxide red as dust), the evidence for carcinogenicity to humans exposed by inhalation to ferric oxide dust (Group 3) was 'inadequate' (IARC, 1987).

Iron oxides and hydroxides are included in the Commission Decision 2006/257/EC[17] establishing an inventory of ingredients authorised in cosmetic products.

2.9. Exposure assessment

2.9.1. Food consumption data used for exposure assessment

2.9.1.1. EFSA Comprehensive European Food Consumption Database

Since 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been populated with national data on food consumption at a detailed level. Competent authorities in the European countries provide EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country (cf. Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011a)). New consumption surveys recently added in the Comprehensive Database were also taken into account in this assessment[18].

The food consumption data gathered by EFSA were collected by different methodologies and thus direct country-to-country comparisons should be interpreted with caution. Depending on the food category and the level of detail used for exposure calculations, uncertainties could be introduced owing to possible underreporting by subjects and/or misreporting of the consumption amounts.

[17]Commission Decision of 9 February 2006 amending Decision 96/335/EC establishing an inventory and a common nomenclature of ingredients employed in cosmetic products. OJ L 97, 5.4.2006, p. 1.
[18] Available online at: http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm

Nevertheless, the EFSA Comprehensive Database represents the best available source of food consumption data across Europe at present.

Food consumption data for infants, toddlers, children, adolescents, adults and the elderly were used for the exposure assessment. For the present assessment, food consumption data were available from 33 different dietary surveys carried out in 19 European countries (Table 5).

Table 5: Population groups considered for the exposure estimates of iron oxides and hydroxides (E 172)

Population	Age range	Countries with food consumption surveys covering more than 1 day
Infants	From 4 months up to and including 11 months of age	Bulgaria, Denmark, Finland, Germany, Italy, UK
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Denmark, Finland, Germany, Italy, Netherlands, Spain, UK
Children[(a)]	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden, UK
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Italy, Latvia, Spain, Sweden, UK
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Romania, Spain, Sweden, UK
The elderly[(a)]	From 65 years of age and older	Austria, Belgium, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Romania, Sweden, UK

(a) The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of the 'elderly' and the 'very elderly' in the Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011a).

Consumption records were codified according to the FoodEx classification system (EFSA, 2011b). Nomenclature from the FoodEx classification system has been linked to the Food Classification System (FCS) as presented in Annex II of Regulation (EC) No 1333/2008, part D, to perform exposure estimates.

2.9.1.2. Food categories selected for the exposure assessment of iron oxides and hydroxides (E 172)

The food categories in which the use of iron oxides and hydroxides (E 172) is authorised were selected from the nomenclature of the EFSA Comprehensive Database (FoodEx classification system food codes), at the most detailed level possible (up to FoodEx level 4) (EFSA, 2011b).

Some food categories are not referenced in the EFSA Comprehensive Database; therefore, no consumption data are available for them and could therefore not be taken into account in the present estimate. This may result in an underestimation of the exposure. The food categories that were not taken into account are described below (in ascending order of the FCS code):

- 01.7.3 Edible cheese rind,
- 01.7.6 Cheese products (excluding products falling in category 16),
- 04.1.1 Entire fresh fruit and vegetables, only as a contrast enhancer for marking citrus fruit, melons and pomegranates,
- 04.2.4.1 Fruit and vegetable preparations excluding compote, only mostarda di frutta,

- 04.2.4.1 Fruit and vegetable preparations excluding compote, only seaweed based fish roe analogues,
- 05.4 Decorations, coatings and fillings, except fruit-based fillings covered by category 04.2.4,
- 06.6 Batters,
- 06.7 Pre-cooked or processed cereals,
- 08.3.3 Casings and coatings and decorations for meat,
- 14.2.4 Fruit wine and made wine,
- 14.2.5 Mead,
- 14.2.7.3 Aromatised wine-product cocktails.

For the food categories 17.1/17.2/17.3 (i.e. food supplements in liquid, syrup-type or chewable form and solid form), it is not possible to differentiate the food supplement forms within the FoodEx classification codes. Therefore, the mean of the usage levels reported for iron oxides and hydroxides (E 172) in food supplements was assigned to these categories.

For flavoured drinks (FCS 14.1.4), the only available use level reported for a niche product (a multivitamin soft drink with sweetener) was assigned to the whole food category and used in the exposure calculations. This results in an overestimation of the exposure.

In total, 12 food categories were not taken into account in the exposure assessment because they are not referenced in the EFSA Comprehensive Database; therefore, no consumption data are available. For 38 food categories, no concentration data were provided to EFSA. Overall, in the current exposure estimates, 39 food categories were not included in the exposure estimates for one or both reasons mentioned above (not referenced in the Comprehensive Database or concentration data were not available), and only 10 out of 49 food categories could be taken into account (see Appendix B).

2.9.2. Exposure to iron oxides and hydroxides (E 172) from their use as food additives

The Panel estimated chronic exposure to iron oxides and hydroxides (E 172) for infants, toddlers, children, adolescents, adults and the elderly. Dietary exposure was calculated by multiplying iron oxide and hydroxide (E 172) concentrations reported in Appendix B for each food category with their respective consumption amount per kilogram of body weight for each individual in the Comprehensive Database. The exposure per food category was subsequently added to derive an individual total exposure per day. These exposure estimates were averaged over the number of survey days, resulting in an individual average exposure per day for the survey period. Surveys with only 1 day per subject were excluded as considered not adequate to assess repeated dietary exposure.

This was carried out for all individuals per survey and per population group, resulting in distributions of individual average exposure per survey and population group (Table 5). Based on these distributions, the mean and 95th percentiles of exposures were calculated per survey for the total population and per population group. High percentile exposure was only calculated for those population groups where the sample size was sufficiently large to allow calculation of the 95th percentile of exposure (EFSA, 2011a). Therefore, in the present assessment, high levels of exposure for infants from Italy and for toddlers from Belgium, Italy and Spain were not included. Thus, for the present assessment, food consumption data were available from 33 different dietary surveys carried out in 19 European countries (Table 5).

Dietary exposure to iron oxides and hydroxides (E 172) from their use as food colours was estimated using the approach adopted by the Panel at its 52nd meeting. This approach is to be followed to assess the exposure as part of the safety assessment of food additives under re-evaluation with the use of the food consumption data available within the EFSA Comprehensive Database, as presented in Table 5, and with the limitations described above. Exposure assessment of iron oxides and hydroxides (E 172) was carried out by the ANS Panel based on (1) maximum reported use levels (defined as the *maximum level exposure assessment scenario*) and (2) reported use levels (defined as the *refined exposure assessment scenario*) as provided to EFSA by industry. These two scenarios are discussed in detail below.

Due to limited information available on the type of iron oxides and hydroxides (yellow, red or black) used in each of the food categories, the exposure estimates for E 172 calculated by the Panel were based on the MLs/reported use levels irrespectively of the type of iron oxide.

2.9.2.1. Maximum level exposure assessment scenario

The regulatory maximum level exposure assessment scenario is based on the MPLs as set in Annex II to Regulation (EC) No 1333/2008. As iron oxides and hydroxides (E 172) are authorised according to QS in almost all food categories, a 'maximum level exposure assessment' scenario was estimated based on the maximum reported use levels provided by industry (Appendix A), as described in the EFSA Conceptual framework (EFSA ANS Panel, 2014).

The exposure estimates derived following this scenario should be considered as the most conservative compared to the estimates derived based on the refined exposure assessment scenarios (Section 2.9.2.2.), as this scenario assumes that a consumer will be continuously (over a lifetime) exposed to iron oxides and hydroxides (E 172) present in food at the maximum reported use levels.

2.9.2.2. Refined exposure assessment scenario

Based on the available dataset, the Panel calculated two refined exposure estimates based on different model populations:

- The brand-loyal consumer scenario, in which it is assumed that a consumer is exposed long term to iron oxides and hydroxides (E 172) present at the maximum reported use level for one food category. This exposure estimate is calculated as follows:
  - by combining food consumption with the maximum reported use level for the main contributing food category at the individual level;
  - by using the mean of the typical reported use levels for the remaining food categories.
- The non-brand-loyal consumer scenario, in which it is assumed that a consumer is exposed long term to iron oxides and hydroxides (E 172) present at the mean reported use levels in food. This exposure estimate is calculated using the mean of the typical reported use levels for all food categories.

Appendix B summarises the concentration levels of iron oxides and hydroxides (E 172) used in the exposure assessment scenarios.

The Panel noted that only 10 out of the 49 food categories in which the use of iron oxides and hydroxides (E 172) is authorised could be taken into account. If, nevertheless, iron oxides and hydroxides (E 172) are used in the remaining 39 food categories for which concentration data were not available, the calculated exposure estimates might result in underestimation of the actual exposure to iron oxides and hydroxides (E 172).

2.9.2.3. Anticipated exposure to iron oxides and hydroxides (E 172)

Table 6 summarises the estimated exposure to iron oxides and hydroxides (E 172) from their use as food additives in six population groups (Table 5) according to the different exposure scenarios (Sections 2.9.2.1 and 2.9.2.2). Detailed results per population group and survey are presented in Appendix C.

Table 6: Summary of anticipated exposure to iron oxides and hydroxides (E 172) from their use as food additives in the maximum level exposure assessment scenario and in the refined exposure scenarios, in six population groups (minimum–maximum across the dietary surveys in mg/kg bw/day)

	Infants (4-11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Maximum level exposure assessment scenario						
Mean	0.1-2.2	0.4-10.5	1.4-9.2	0.7-4.5	0.3-2.4	0.3-2.3
High level (95th percentile)	0.2-11.7	1.7-26.9	4.0-21.9	2.2-11.3	1.3-7.5	1.0-6.8
Refined exposure assessment scenario						
Brand-loyal scenario						
Mean	0.1-2.0	0.4-8.9	1.2-7.8	0.6-3.9	0.3-2.3	0.3-2.2
High level (95th percentile)	0.2-10.5	1.6-23.1	3.4-19.2	1.7-9.9	1.2-7.3	1.0-6.4
Non-brand-loyal scenario						
Mean	0.03-0.8	0.2-3.7	0.5-3.1	0.2-1.6	0.1-0.8	0.1-0.8
High level (95th percentile)	0.1-4.2	0.7-9.5	1.3-7.6	0.7-4.0	0.5-2.4	0.4-2.3

2.9.3. Main food categories contributing to exposure to iron oxides and hydroxides (E 172) using the maximum level exposure assessment scenario

Table 7: Main food categories contributing to exposure to iron oxides and hydroxides (E 172) using maximum usage levels (> 5 % to the total mean exposure) and number of surveys in which each food category is contributing

FCS category no.	FCS food category	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[(a)]					
03	Edible ices	-	5.3-12.4 (3)	5.0-14.8 (4)	5.4-7.6 (2)	5.7 (1)	6.5 (1)
05.2	Other confectionery including breath freshening microsweets	-	11.3 (1)	6.4-62.4 (5)	5.8-73.8 (6)	5.1-18.9 (4)	5.2-10.0 (2)
05.3	Chewing gum	-	-	-	5.8-7.0 (2)	6.1 (1)	-
07.2	Fine bakery wares	28.0-98.8 (4)	37.4-96.0 (10)	39.4-94.4 (17)	37.7-92.7 (16)	54.6-92.9 (17)	52.2-94.7 (14)
14.1.4	Flavoured drinks	-	-	7.7 (1)	11.6 (1)	5.7-8.0 (3)	-
15.1	Potato-, cereal-, flour- or starch-based snacks	-	-	-	-	8.4 (1)	-
16	Desserts excluding products covered in categories 1, 3 and 4	23.7-68.0 (3)	6.5-51.4 (8)	8.8-44.1 (12)	6.1-31.0 (11)	5.1-33.0 (12)	6.8-42.2 (10)

FCS category no.	FCS food category	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[a]					
17	Food supplements as defined in Directive 2002/46/EC excluding food supplements for infants and young children	98.1-100.0[b] (2)	27.4 (1)	12.0 (1)	5.9 (1)	5.7-10.7 (4)	7.8-9.5 (2)

(a) The total number of surveys may be greater than the total number of countries listed in Table 5, as some countries submitted more than one survey for a specific population.
(b) The very high contribution of food supplements to the exposure of E 172 observed in infants is due to food supplements being the only source of exposure to E 172 for this population in two countries.

2.9.4. Main food categories contributing to exposure to iron oxides and hydroxides (E 172) using the refined exposure assessment scenarios

Table 8: Main food categories contributing to exposure to iron oxides and hydroxides (E 172) using the brand-loyal refined exposure scenario (> 5 % to the total mean exposure) and number of surveys in which each food category is contributing

FCS category no.	FCS food category	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[a]					
03	Edible ices	-	6.1-13.5 (3)	5.5-16.1 (4)	5.3-9.1 (3)	6.6 (1)	7.0 (1)
05.2	Other confectionery including breath freshening microsweets	-	6.3 (1)	15.9-65.2 (2)	5.6-78.1 (3)	10.9-12.1 (2)	6.0 (1)
07.2	Fine bakery wares	25.9-98.9 (4)	36.9-96.8 (10)	44.5-95.7 (17)	42.3-93.5 (16)	58.1-93.6 (17)	50.3-94.7 (14)
14.1.4	Flavoured drinks	-	-	9.3 (1)	13.9 (1)	5.8-9.2 (3)	-
15.1	Potato-, cereal-, flour- or starch-based snacks	-	-	-	-	8.6 (1)	-
16	Desserts excluding products covered in categories 1, 3 and 4	24.3-70.5 (3)	6.9-61.5 (8)	7.3-47.9 (12)	7.2-32.4 (10)	5.4-33.9 (11)	6.1-45.9 (9)
17	Food supplements as defined in Directive 2002/46/EC excluding food supplements for infants and young children	98.2-100.0[b] (2)	25.4 (1)	9.6 (1)	-	5.4-11.1 (3)	5.3-8.2 (2)

(a) The total number of surveys may be greater than the total number of countries listed in Table 5, as some countries submitted more than one survey for a specific population.
(b) The very high contribution of food supplements to the exposure of E 172 observed in infants is due to food supplements being the only source of exposure to E 172 for this population in two countries.

Table 9: Main food categories contributing to exposure to iron oxides and hydroxides (E 172) using the non-brand-loyal refined exposure scenario (> 5 % to the total mean exposure) and number of surveys in which each food category is contributing

FCS category no.	FCS food category	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys)[a]					
03	Edible ices	7.0 (1)	6.9-30.4 (4)	5.6-45.7 (17)	5.2-22.6 (13)	5.4-15.3 (6)	8.9-18.5 (2)
05.2	Other confectionery including breath freshening microsweets	-	-	7.1-23.4 (2)	7.2-37.2 (2)	5.4-6.1 (2)	-
05.3	Chewing gum	-	-	-	7.1 (1)	-	-
07.2	Fine bakery wares	22.3-96.4 (4)	29.2-91.5 (10)	29.1-87.1 (17)	26.8-81.0 (16)	43.1-85.0 (17)	45.4-87.2 (14)
14.1.4	Flavoured drinks	7.3 (1)	5.3-9.0 (3)	5.3-19.2 (8)	6.1-28.0 (10)	6.2-21.6 (7)	8.8-11.0 (3)
15.1	Potato-, cereal-, flour- or starch-based snacks	6.7 (1)	-	5.3-6.5 (3)	5.9-7.8 (4)	20.3 (1)	8.9 (1)
16	Desserts excluding products covered in categories 1, 3 and 4	25.3-69.2 (3)	5.9-56.1 (8)	5.0-47.3 (13)	7.3-32.4 (11)	5.1-35.0 (13)	8.2-46.6 (10)
17	Food supplements as defined in Directive 2002/46/EC excluding food supplements for infants and young children	96.4-100.0[b] (2)	25.5 (1)	14.1 (1)	9.3 (1)	6.3-12.2 (4)	9.5-11.2 (2)

FCS: Food Categorisation System.

(a) The total number of surveys may be greater than the total number of countries listed in Table 5, as some countries submitted more than one survey for a specific population.

(b) The very high contribution of food supplements to the exposure of E 172 observed in infants is due to food supplements being the only source of exposure to E 172 for this population in two countries.

2.9.5. Uncertainty analysis

Uncertainties in the exposure assessment of iron oxides and hydroxides (E 172) have been discussed above. In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2006), the sources of uncertainties summarised in Table 10 have been considered.

Table 10: Qualitative evaluation of influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction[(a)]
Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/–
Use of data from food consumption survey of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Correspondence of reported use levels to the food items in the EFSA Comprehensive Food Consumption Database: uncertainties to which precise types of food the levels refer to	+/–
Food categories selected for the exposure assessment: exclusion of food categories due to missing FoodEx linkage (12/49 food categories)	–
Food categories excluded in the exposure assessment: concentration data not available for certain food categories, which could not be included in the exposure estimates (38/49 food categories)	–
Reported use levels: - use levels considered applicable for all items within the entire food category - information on the type of iron oxide (yellow, red, black) used in foods not always available, overall exposure estimated irrespective of the type of iron oxide	 + +
Maximum level exposure assessment scenario: the maximum reported use levels were considered for the food categories authorised at QS	+
Refined exposure assessment scenarios: exposure calculations based on the maximum or mean reported use levels from industries	+/–
Uncertainty in possible national differences in use levels of food categories	+/–

(a): Uncertainties with potential to cause overestimation of exposure are indicated by '+'; uncertainties with potential to cause underestimation of exposure are indicated by '–'.

Overall, the Panel considered that the uncertainties identified would result in an underestimation of the actual exposure to iron oxides and hydroxides (E 172) used as food additives in European countries.

3. Biological and toxicological data

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations and additional literature that has become available since then. The toxicity of iron oxides and hydroxides has been evaluated previously by JECFA in 1974, 1978 and 1980 (JECFA, 1974, 1975, 1978, 1980) and the SCF in 1975. It was also briefly reviewed by TemaNord (2002). The present opinion briefly reports the major studies evaluated in these opinions and describes any additional data in more detail.

No new toxicological or biological information was submitted to the Panel for the re-evaluation of iron oxides and hydroxides (E 172) following EFSA public calls for data. The Panel noted that not all of the original studies on which previous evaluations were based were available for the present re-evaluation. A literature search was conducted on the most commonly available online databases for toxicological and biological information (PubMed, Science Direct, Toxline and Web of Knowledge) to cover recent published literature on iron oxides and hydroxides (E 172).

The Panel noted that in many studies the particle size distribution of iron oxides was not stated.

3.1. Absorption, distribution, metabolism and excretion (ADME)

3.1.1. Absorption, distribution and excretion of iron oxide microparticles

As reported previously (Emerton, 2008), yellow, red and black iron oxides are known to be insoluble in water and they can only be solubilised in concentrated mineral acids, which are not associated with foods. Iron oxides are negatively charged at physiological pH (Suh et al., 2009). Kraemer (2004) indicated that iron oxides are partially soluble in acidic media containing chelators. Reviewing the properties and toxicology of iron oxides, Rowe (1984) reported the low solubility of yellow, red and

black iron oxides in gastric (0.03–0.25 %, 0.1–0.76 % and 0.9–1.25 % of the dose, respectively) and intestinal (0.008–0.035 % for all oxides) juices.

3.1.1.1. Animal study

Singh et al. (2013) compared the tissue distributions of microsized red iron oxide (Fe_2O_3-bulk, 2.15 µm) and nanosized red iron oxide (Fe_2O_3-30 nm) in female Wistar rats. The rats were treated orally with single doses of 500, 1 000 or 2 000 mg/kg bw of nano and microsized red iron oxide. At 6, 24, 48 and 72 h after treatment, the distribution of iron (Fe) was measured in the liver, spleen, kidney, heart, brain, bone marrow, urine and faeces by atomic absorption spectrophotometry after an overnight digestion of the biological samples in nitric acid. A small percentage of iron was absorbed in the gastrointestinal tract when microsized red iron oxide was orally administered. Iron was found in the tissues after a single oral dose and unabsorbed iron was excreted via faeces. When red iron oxide was administered orally in the nano form (30 nm), particles easily passed across the gastrointestinal barrier and accumulated in the organs and tissues where higher iron concentrations were measured compared with microsized red iron oxide particles. The incorporation of iron in the various tissues was in the range of 0.01–2.3 % in the microsized red iron oxide-treated groups and 0.2–9.4 % in the groups treated with nano red iron oxide. Regarding excretion of iron, after oral administration of both nanosized and microsized red iron oxide, small amounts of iron were excreted via urine, while most were excreted via faeces. However, in rats treated with microsized Fe_2O_3, the urinary concentrations of iron were not significantly different from the control values, and were much lower than in the rats treated with the nanoparticulate material. The Panel considered that in this study, the iron measured in all biological samples should correspond to both particulate and ionised iron due to the acidic digestion of these samples carried out before the spectrophotometric measurement of iron.

3.1.1.2. Human studies

In its evaluation of iron oxides (and hydrated iron oxides), the SCF (1975) mentioned that there was information (reference not provided) showing that only 1 % of the colour was likely to become solubilised in the human intestinal tract. JECFA (1980) reported that studies on the bioavailability of iron from iron oxides and hydroxides suggest that iron from ferric oxide is less biologically active than iron from other sources.

Derman et al. (1977) measured the absorption of iron added to maize-meal porridge in 116 volunteer multiparous Indian women using the radio-Fe erythrocyte utilisation method. Meals were consumed after overnight fasting and no food or drink was consumed for 4 hours after the meal had been eaten. The mean absorption of iron (Fe) from maize-meal porridge was very low (3.8 % of the dose). The addition of 50 or 100 mg ascorbic acid to maize-meal porridge caused approximately a 10-fold increase in Fe absorption. When contaminating Fe (2.5 mg) in the form of labelled rust (red iron oxide; Fe_2O_3) or ferric hydroxide (yellow iron oxide; FeO(OH)) (particle sizes not indicated) was added to maize-meal porridge, iron was poorly absorbed (mean values were 0.01 % and 1.5 % respectively). The addition of 100 mg ascorbic acid increased the mean Fe absorption rates to 0.5 % and 6.7 %, from Fe_2O_3 and FeO(OH) respectively. Yellow iron oxide (FeO(OH) was found to be absorbed about half as well as the intrinsic Fe present in maize-meal porridge. The authors concluded that ascorbic acid is capable of enhancing Fe absorption from a cereal source and might be expected to facilitate the absorption of at least some forms of Fe that may contaminate food.

Using a similar approach, the same authors (Derman et al., 1982) compared the absorption of iron from ferritin and from yellow iron oxide (FeO(OH)) (particle size not indicated) in 35 multiparous women when fed in water, in maize porridge with and without 100 mg ascorbic acid. The mean absorption for 3 mg ferritin or yellow iron oxide (FeO(OH)) was 0.7 and 2.4 %, respectively. When 100 mg ascorbic acid were added in the porridge, absorption was increased (12.1 and 10.5 % for ferritin and yellow iron oxide (FeO(OH)), respectively). The authors concluded from these results that the fraction of iron in ferritin or ferric hydroxide that enters the pool of non-haem dietary iron is profoundly influenced by the nature of the diet.

The JECFA evaluation of iron (1983) also reported various observations of iron intake in humans. In particular, it reported a scenario in Ethiopia where contamination of cereal grain with iron-rich soil may result in a mean iron intake of 500 mg/day in the form of an unidentified complex of iron oxides and hydroxides (Hofvander, 1968). According to the author, these forms of iron would not be absorbed, as this intake had never been reported to result in siderosis.

3.1.2. Distribution of black iron oxide ($FeO \cdot Fe_2O_3$) nanoparticles

There is no information on the distribution of microparticles of black iron oxide ($FeO \cdot Fe_2O_3$) in animals or humans. However, various recent studies investigated the pharmacokinetics of black iron oxide nanoparticles, which are used in medical applications such as magnetic resonance imaging, magnetic hyperthermia, and targeted drug and gene delivery. These magnetic particles generally consist of nanoparticles of black iron oxide measuring 10–50 nm in diameter.

Many reports described the tissue distribution of ^{59}Fe-black iron oxide ($FeO \cdot Fe_2O_3$) nanoparticles following intravenous injection to rats (Weissleder et al., 1989; Okon et al., 1994; Bourrinet et al., 2006; Jain et al., 2008). These studies were considered as not relevant for the assessment of black iron oxide used as a food additive because of the intravenous route of administration.

Wang et al. (2010) explored the tissue distribution of iron from nanosized black iron oxide ($FeO \cdot Fe_2O_3$, average particle size 20 nm) in ICR mice receiving intragastric administration of a single dose of 600 mg/kg bw of nanoparticles and observed over a period of 10 days. The distribution of iron from black iron oxide nanoparticles in tissues was measured using atomic absorption spectrophotometry. Iron was distributed in the peripheral blood, heart, lungs, kidneys, brain, stomach, small intestine and bone marrow, the majority of iron being distributed in the liver and the spleen. Levels in brain tissue were higher in the treated group than in the control group, indicating that iron from black iron oxide nanoparticles can penetrate the blood–brain barrier. The Panel noted that the percentage of absorption of iron from these nanoparticles was not mentioned in this study.

Conclusion on absorption, distribution and excretion

Overall, 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 %) was described in similar populations. In these human studies, the addition of ascorbic acid increased by 5–50 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 ($FeO \cdot Fe_2O_3$).

3.2. Toxicological data

The Panel noted that in most of the studies in the biological and toxicological database included in the present opinion, there was no specification of the test material, including an indication of the particle size.

3.2.1. Acute oral toxicity

Unspecified iron oxides

The JECFA (1975) evaluation reported oral LD_{50} values for unspecified 'iron oxide' of 15 g/kg bw in rats and mice (Steinhoff, 1972).

Yellow iron oxide ($FeO(OH) \cdot H_2O$)

A LD_{50} above 10 g/kg was reported for yellow iron oxide (FeO(OH)) in rats (Bayer, 1977a).

Red iron oxide (Fe_2O_3)

An unpublished study (Bayer, 1977b) reported that the oral LD_{50} for red iron oxide (Fe_2O_3) was above 10 g/kg bw in rats. In another rat study with red iron oxide, the LD_{50} was above 5 g/kg bw (Ramm, 1986).

Kumari et al. (2013) compared the size-, dose- and time-dependent effects, after acute oral exposure to microparticles and nanoparticles of red iron oxide (Fe_2O_3), on various biochemical enzyme activities in a female Wistar rat model. In this study complying with OECD TG 421 (OECD, 1995), rats were exposed to three different single doses (500, 1 000 and 2 000 mg/kg bw) of nano red iron oxide (30 nm, NP 98 %) or bulk red iron oxide (<5 µm, 99 %) and compared to a control group. No effect was observed on growth, behaviour and nutritional performance of animals. In all groups treated with red iron oxide (Fe_2O_3) nanoparticles, statistically significant inhibition (24–28 %) of acetylcholinesterase in red blood cells and a 80 % inhibition of total Na^+ K^+, Mg^{2+} and Ca^{2+}-ATPases in brain were observed. Aspartate aminotransferase, alanine aminotransferase and lactate dehydrogenase activities were increased in the liver of the same animals. Moreover, these enzymes were found to be increased in the serum and reduced in the kidney of animals receiving the highest or the two highest doses of nanosized red iron oxide. By contrast, microsized red iron oxide did not result in any significant changes in these biochemical parameters. According to the authors, this study suggests that exposure to nanosized particles at acute doses may cause adverse changes in animal biochemical profiles.

Black iron oxide ($FeO{\cdot}Fe_2O_3$)

A LD_{50} above 10 g/kg bw was reported for black iron oxide ($FeO{\cdot}Fe_2O_3$) in rats (Bayer, 1977c).

3.2.2. Subacute and subchronic toxicity

3.2.2.1. Subacute toxicity

Yellow ($FeO(OH){\cdot}H_2O$) and black ($FeO{\cdot}Fe_2O_3$) iron oxides

No data available.

Red iron oxide (Fe_2O_3), including nanoparticles

A study in rats fed diets containing 700, 1 160, 1 610 or 2 060 mg red iron oxide/kg diet (equivalent to 83, 137, 190 or 243 mg (Fe_2O_3)/kg bw/day) for 21 days was mentioned in the IUCLID dataset document (EC, 2000a). No toxic effects were reported in these animals and there was no increase of liver non-haemoglobin iron content.

In a subacute repeated dose oral toxicity study complying with OECD TG 408 (OECD, 1998), groups of ten female Wistar rats received by gavage 0 (controls), 30, 300 or 1 000 mg/kg bw/day of red iron oxide nanoparticles (Fe_2O_3-30 nm) or microsized red iron oxide (Fe_2O_3-Bulk, <5 µm, 99 %), for 28 days (Kumari et al., 2012). As measured by TEM characterisation, the mean of red iron oxide nanoparticles was 29.75 ± 1.87 nm. At the end of the treatment period, animals were sacrificed, serum and organs were collected. Biochemical enzymes were measured in erythrocytes, serum, brain, liver and kidney of rats. Histopathology was carried out in the liver, kidney, spleen, heart and brain of all animals. No decrease in body weight, no change in feed intake, nor any adverse sign, symptoms or mortality were observed in rats exposed to microsized red iron oxide or up to 300 mg/kg bw/day of red iron oxide nanoparticles. However, rats treated with the high dose of red iron oxide nanoparticles (1 000 mg/kg bw/day) showed reduced body weight and feed intake accompanied by severe toxic symptoms such as dullness, irritation and moribund condition. In this group of animals, several disturbances were observed in biochemical parameters, and histopathology results showed necrosis in the liver, kidney and spleen. By contrast, microsized red iron oxide did not induce any adverse effects in either biochemical parameters or histopathology in the treated rats, with the exception of a significant decrease (25 %) in lactate dehydrogenase activity in the kidneys of rats given the highest dose. However, the Panel noted that no significant change in plasma lactate dehydrogenase activity

was observed in this group of animals and therefore did not consider the decreased lactate dehydrogenase activity in the kidney adverse. Overall, these results indicate 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 no-observed-adverse effect (NOAEL) for microsized red iron oxide of 1 000 mg/kg bw/day, the highest dose tested, and a NOAEL of 300 mg/kg bw/day for nanosized (30 nm) red iron oxide.

3.2.2.2. Subchronic toxicity

No subchronic toxicity studies by oral administration of microsized yellow iron oxide (FeO(OH)), red iron oxide (Fe_2O_3) or black iron oxide ($FeO{\cdot}Fe_2O_3$) were available.

Red iron oxide (Fe_2O_3) nanoparticles

Recently, Yun et al. (2015) compared the subchronic toxicity of various orally administered nanoparticles. Besides silicon dioxide and silver, these authors used red iron oxide (Fe_2O_3) obtained from NanoAmor Co, Ltd (not further specified). These nanoparticles were characterised by α-form, primary size of 60 nm, hydrodynamic size of 117.9 ± 78.0 nm and a zeta potential of 13.6 mV. In a preliminary acute toxicity study, these nanoparticles were found to be without toxic effects in rats receiving 1 000 mg/kg bw/day for 14 days. The 13-week repeated oral toxicity study was performed according to the OECD TG 408 (OECD, 1998). Sprague-Dawley rats (12 per sex per group) received daily doses of 250, 500 or 1 000 mg/kg bw/day for 13 weeks by gavage, in the form of an aqueous suspension. Urinalysis, haematology and serum biochemistry were determined at the end of the treatment period. Gross findings, organ weights, histopathological assessment and distribution and excretion of iron were analysed for each animal. Fe_2O_3 nanoparticles had no significant effects on body weight, mean daily food and water consumption, when compared with control groups. There were no treatment-related changes in haematological, serum biochemical parameters or histopathological observations. Some changes in organ weights were observed: decreases in weight of pituitary gland and liver and increases in weight of adrenal gland and testis. According to the authors, 'these changes were sporadic without dose-dependent trends, indicating that they were not considered toxicologically relevant'. In blood and all tissues tested, including liver, kidney, spleen, lung and brain, the concentration of Fe 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. Although not statistically significant, the concentrations of Fe in the faeces of treated animals were found to be higher than those of the control groups. 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 of 1 000 mg/kg bw/day, the highest dose tested in rats receiving Fe_2O_3 nanoparticles by gavage.

Black iron oxide ($FeO{\cdot}Fe_2O_3$)

No studies are available on black iron oxide by oral administration.

3.2.3. Genotoxicity

No genotoxicity studies were mentioned in previous evaluation reports on iron oxides and hydroxides from the SCF (1975) or JECFA (1974, 1975 and 1980).

3.2.3.1. Genotoxicity of yellow iron oxide ($FeO(OH){\cdot}H_2O$)

No data available.

3.2.3.2. *In vitro* assays on red iron oxide (Fe_2O_3) and black iron oxide ($FeO{\cdot}Fe_2O_3$)

In a screening study on 228 pesticides, red iron oxide (Fe_2O_3) was tested for mutagenicity in a bacterial reverse mutation assay using *Salmonella typhimurium*, both in the absence and presence of S9 metabolism (Moriya et al., 1983). Red iron oxide was only assayed in tester strains TA98 and TA100 and was reported to be negative.

Garry et al. (2004) reported results in an *in vitro* alkaline comet assay to measure DNA damage in four different cell types isolated from Sprague-Dawley rats at 1, 2, 4, 8 and 24 hours after *in vitro* treatment with red iron oxide (Fe_2O_3) or benzo(a)pyrene (B[a]P) or B[a]P coated onto red iron oxide. The results obtained for red iron oxide indicated absence of DNA damage at a dose level of 10µg/mL in all the different cell types at any sampling time employed. Treatments with B[a]P or B[a]P coated onto red iron oxide did not induce significant DNA damage in alveolar macrophages. On the contrary, significant increase in DNA damage was observed in lymphocytes, hepatocytes and lung cells, where the effect of B[a]P coated onto red iron oxide was more pronounced than B[a]P alone. The Panel noted that this study was designed to evaluate the potentiating effect of iron oxide on benzo(a)pyrene genotoxicity, and thus the negative result reported with iron oxide alone has limited significance, as only a single dose level at a relatively low concentration was tested.

In a study by Karlsson et al. (2008) which aimed to investigate the genotoxicity of subway particles, commercially available black iron oxide ($FeO{\cdot}Fe_2O_3$), particle size 1–3 µm, and red iron oxide (Fe_2O_3), particle size < 1 µm, were assessed for their genotoxic potential in an *in vitro* alkaline comet assay using the human lung epithelial cell line A549. The results obtained indicated that both black iron oxide and red iron oxide induced statistically significant increases in DNA damage (DNA single-strand breaks and alkali labile sites). Furthermore, the ability to cause oxidative DNA damage was assessed using the comet assay in combination with the enzyme formamidopyrimidine DNA glycosylase (FPG). The results obtained showed that subway particles induced significant increases in FPG sites compared to the untreated control, while black iron oxide particles did not.

In a following study, Karlsson et al. (2009) compared the toxicity of nano- and micrometre particles of red iron oxide (Fe_2O_3), average particle sizes 29 nm and < 1 µm, respectively, and black iron oxide ($FeO{\cdot}Fe_2O_3$), average particle sizes 20–30 nm and 0.5 µm, respectively, in the human alveolar type II-like cell line A549. Genotoxic potential was investigated by means of alkaline comet assay to detect DNA breakage. Analysis of oxidative DNA lesions, mainly oxidised purines, was performed applying to comet assay the FPG enzyme. When A549 cells were treated with nano- and micrometre particles of red iron oxide or black iron oxide for 4 hours at 40 and 20 µg/cm^2, statistically significant increases in DNA damage compared to untreated controls were only observed with the micrometre particle. On the contrary, for oxidative DNA damage, only nanoparticles of black iron oxide at 40 µg/cm^2 for 4 hours caused significant increases in oxidised purines. None of the nanoparticles caused a significant increase in oxidised purines following exposure to 20 µg/cm^2 for 4 hours.

The DNA damaging potential of red iron oxide (Fe_2O_3) (particle size < 100 nm), was investigated in an *in vitro* comet assay using human lung fibroblasts (IMR-90) and human bronchial epithelial (BEAS-2B) cells (Bhattacharya et al., 2009). The results obtained indicated that red iron oxide induced significant DNA breakage in both the IMR-90 cell line at 10 and 50 µg/cm^2 and the BEAS-2B cell line at 50 µg/cm^2 in the presence of an adequate reduction of cell viability (50 % and 40 % of the concurrent negative controls, respectively) following 24-hour treatment. The genotoxic effects were supposed to be induced by generation of reactive oxygen species (ROS) which requires reducing conditions within the cells to convert Fe(III) to Fe(II). This was confirmed in an acellular system.

In an *in vitro* cytochalasin-block micronucleus assay (Pfaller et al., 2010), red iron oxide (Fe_2O_3) (particle size 7.3 nm), was assessed for its genotoxicity in peripheral blood of two different healthy donors at concentrations of 6.0×10^{10}, 6.0×10^{11} and 6.0×10^{12} nanoparticles/mL. The indices of genotoxicity and cytotoxicity were defined as the frequency of binucleate micronucleated leukocytes (BNMN) and the cytokinesis block proliferation index (CBPI), respectively. The results obtained showed that treatments did not induce any cytotoxic effect and small, non-significant increases in the frequencies of micronucleated binucleate leukocytes were observed.

Könczöl et al. (2011) studied the genotoxicity of black iron oxide ($FeO{\cdot}Fe_2O_3$) in *in vitro* comet and cytokinesis block micronucleus assays using the human lung cells (A549). Four size fractions of black iron oxide were investigated: bulk black iron oxide (0.2–10 µm), respirable fraction (2–3 µm), alveolar fraction (0.5–1.0 µm) and nanoparticles (20–60 nm). The results obtained in the comet assay indicated

that all black iron oxide fractions induced dose-related increases in DNA damage following 4-hour treatments. Significantly increased DNA migration, as measured by Olive tail moment (OTM) and tail intensity (TI), was observed for all fractions at 50 µg/cm^2. Bulk (0.2–10 µm) and 20–60 nm nanoparticles showed the highest DNA migration. The DNA-damaging effect was reduced by simultaneous addition of 1 mmol/L *N*-acetyl-cysteine (NAC) to A549 cells or by pretreatment with 100 µmol/L butylated hydroxyanisole (BHA) at two different concentrations of nanoparticles (20–60 nm). The inhibitory effect of both ROS scavenging agents was higher for the cells pre-treated with BHA compared to those incubated with NAC. In the micronucleus test, the most pronounced and significant effects were observed in the cells treated with bulk black iron oxide (0.2–10 µm), alveolar fraction (0.5–1.0 µm) and nanoparticles (20–60 nm). In the case of bulk black iron oxide, increases were also dose-dependent and reached the maximum at 100 µg/cm^2. The alveolar fraction (0.5–1.0 µm) showed an increase in micronucleated binucleate cells at 50 µg/cm^2, increasing further at 100 µg/cm^2. For nanoparticles (20–60 nm), a significant increase of micronucleated binucleate cells was observed at 10 µg/cm^2, reaching the maximum at 100 µg/cm^2. The respirable fraction (2–3 µm) showed the lowest induction of micronucleated binucleate cells. Analysis of cytokinesis block proliferation index (CBPI) did not show significant reduction in any of the samples investigated. Simultaneous addition of the ROS scavenger NAC at 1 mmol/L together with two concentrations of nanoparticles (20–60 nm) to A549 cells decreased micronucleus formation almost to the level of the untreated control.

Magdolenova et al. (2011) assessed the genotoxicity of black iron oxide ($FeO{\cdot}Fe_2O_3$), particle size 9 µm, uncoated and coated with oleic acid in the human lymphoblastoid cell line TK6 using the alkaline comet assay modified with lesion-specific endonuclease FPG for detection of DNA strand breaks and oxidised bases. Results obtained indicate that uncoated black iron oxide did not cause cytotoxic and genotoxic effects following 2 and 24-hour treatment in the study concentration range (0.6–75 µg/cm^2). However, the data were poorly reported. Therefore, the reliability of the results cannot be assessed.

In the study by Bhattacharya et al. (2012), the toxicological effects of nanosized red iron oxide (Fe_2O_3, d < 100 nm) and microsized red iron oxide (d < 5 µm) were investigated in human lung cells. Potential DNA damage was also investigated by means of comet assay in non-transformed human lung fibroblasts IMR-90, as well as in SV40 virus-transformed human lung epithelial cells (BEAS-2B). Significant increases in DNA breakage were only found at high dose levels (> 50 µg/mL), where cell viability was markedly reduced. The nanoscale particles were slightly more effective in causing cyto- and genotoxicity as compared with their microscale counterparts. Both types of particles induced intracellular generation of ROS.

Using Syrian hamster embryo (SHE) cells, Guichard et al. (2012) compared the *in vitro* cytotoxicity and genotoxicity of commercially available nanosized and microsized black iron oxide ($FeO{\cdot}Fe_2O_3$) and red iron oxide (Fe_2O_3) particles. Induction of DNA damage and clastogenicity were investigated by means of alkaline comet assay and micronucleus test, respectively. Results obtained showed no genotoxic activity for both nanosized and microsized black and red iron oxide particles.

In addition to the published papers described above, two unpublished GLP studies on *in vitro* genotoxicity testing of black iron oxide, retrieved from the European Chemicals Agency (ECHA) database, were evaluated:

Thum (2008) evaluated the clastogenic potential of black iron oxide powder (92.3 % purity, particle size not specified) in a chromosomal aberration assay in V79 cells. As the test material was insoluble in any solvent, it was sonicated in dimethyl sulfoxide (DMSO) to achieve a fine dispersion. Cells were treated with 6.25, 12.5 and 25 µg/mL black iron oxide, either with S9 or without S9; treatments lasted 4 hours with S9 (with harvest at 18 and 30 hours), and 18 and 30 hours without S9. Chromosomal aberrations were scored in 100 metaphases in each of the duplicate cultures. Precipitation of pigment in culture medium was observed at the top dose. No biologically relevant increase in cells with

structural or numerical chromosomal aberrations was detected. Mild cytotoxicity (60 % of survival at high dose) was only observed in the presence of S9.

Entian (2008) evaluated the mutagenic activity of black iron oxide powder (92.3 % purity, particle size unspecified) in a forward mutation assay at the *hprt* locus in V79 cells. As the test material was insoluble in any solvent, it was sonicated in DMSO to achieve a fine dispersion. Cells were treated with 6, 9, 12, 18, 24 and 36 µg/mL, with and without S9. Precipitation in culture medium was observed at 18 µg/mL and above. No decrease in survival and in relative population growth and no increase in mutant frequency above the negative control were observed in treated cultures, either with or without S9.

3.2.3.3. *In vivo* assays on red iron oxide (Fe_2O_3)

Garry et al. (2003) evaluated the genotoxicity of red iron oxide (Fe_2O_3) in an *in vivo* unscheduled DNA synthesis (UDS) assay in hepatocytes and lung cells of rats 24 hours after intratracheal administration of a single dose of red iron oxide at approximately 3.75 mg/kg, of benzo(a)pyrene (B[a]P) at approximately 3.75 mg/kg or of B[a]P (0.75 mg) coated on haematite particles (0.75 mg). For red iron oxide alone, results obtained indicated that the test compound did not significantly increase UDS in both lung and liver cells compared to the negative control group level. The Panel noted that this study was designed to evaluate the potentiating effect of iron oxide on benzo(a)pyrene genotoxicity, and thus the negative result reported with iron oxide alone has limited significance, as only a single dose level at a relatively low concentration was tested. Moreover, the intratracheal route of administration used in this study is not relevant for risk assessment following oral exposure.

Singh et al. (2013) assessed both the genotoxicity and tissue distribution (see section 3.1.1.1) of nano red iron oxide (Fe_2O_3-30 nm) or microsized red iron oxide (bulk-Fe_2O_3) in female albino Wistar rats treated orally with single doses of 500, 1 000 or 2000 mg/kg bw. Potential DNA breakage was evaluated by comet assay in peripheral blood cells at 6, 24, 48 and 72 hours from treatment. Induction of micronuclei was evaluated in peripheral blood and bone marrow cells at 48 and 72, and 24 and 48 hours from treatment, respectively. Chromosomal aberrations were investigated in bone marrow cells at 18 and 24 hours from treatment. Results obtained indicated that both nanosized and bulk red iron oxide did not show any genotoxic activity, although no adequate toxicity was achieved in the target organs (e.g. decrease of mitotic indices, shift in the ratio polychromatic to normochromatic erythrocytes). However, the presence of red iron oxide nanoparticles in bone marrow was demonstrated (see Section 3.1.1.1). The study essentially meets the requirements of OECD TG 474 and 475 (OECD, 1997a, b) for induction of micronuclei and chromosomal aberrations, respectively, and internationally recognised protocols for comet assay. The authors conclude that under the reported experimental conditions, both nanosized and bulk red iron oxide did not show genotoxic activity. The Panel agreed with this conclusion.

Overall, the Panel considered that the database on genotoxic effects of red iron oxides showed DNA breaking activity of both nano and microsized particles in some studies in cultured mammalian cells *in vitro*, which were not confirmed by other *in vitro* studies, and a negative outcome in a thoroughly conducted *in vivo* study (Singh et al., 2013) with nano and microsized red iron oxide for induction of micronuclei and chromosomal aberrations in peripheral blood and bone marrow cells. Induction of DNA breakage was also not increased in peripheral blood cells. On this basis, the Panel considered that there is no concern with respect to systemic genotoxicity of orally administered red iron oxide. However, site-of-contact effects (gastrointestinal tract) were not addressed in this study. This aspect should be investigated with red iron oxide meeting the specifications of the food additive for a thorough genotoxicity assessment in order to rule out any genotoxic concern.

The Panel noted that the redox state of iron oxide nanoparticles is a critical feature, which may also affect larger (i.e. non-nano) particles. Thus, read-across from red iron oxide (which contains iron(III)) to black iron oxide (which contains iron(II) and iron(III)) should not be performed. Therefore,

additional *in vivo* data on black iron oxide are required to clarify the relevance of positive results observed in three *in vitro* comet assays and one *in vitro* micronucleus assay.

The Panel also noted that there are no data regarding the genotoxicity of yellow iron oxide (FeO(OH)). In principle, as this iron oxide contains only iron(III), it would be covered by the conclusion drawn for red iron oxide. However, the Panel noted that in addition to a difference in the shape of particles, the particle size distribution of yellow iron oxide shows a larger fraction of nanosized particles compared to red and black iron oxides, and thus read-across should not be performed.

3.2.4. Chronic toxicity and carcinogenicity

Unspecified iron oxide

The JECFA (1980) evaluation reported that iron oxide (unspecified compound) in the diet at levels up to 10 g/kg did not result in adverse effects in dogs and cats (no further details reported). These unpublished studies were more fully described in the JECFA evaluation report on iron (1983). In an unpublished study from Carnation Co. (1967), ten dogs were fed on diets containing iron oxide colourant from 1 to 9 years at about 570 mg/lb (equivalent to 1.25 g/kg diet, 0.312 mg/kg bw/day[19]). Daily consumption was estimated at 428 mg/dog. Two Labradors, fed for 1 year, had loose faeces; otherwise, no adverse effects were observed. In a study from Ralston Purina Cat Care Center (1968), no adverse effects were reported in cats maintained on diets containing 1 900 mg/kg diet (475 mg/kg bw/day) of iron from iron oxide (equivalent to 0.27 % iron oxide) for periods of 2–9 years.

The Panel noted that this study was requested to the Food and Drug Administration (FDA) but was not available, and therefore could not be evaluated.

Red iron oxide (Fe_2O_3), yellow iron oxide ($FeO(OH)\cdot H_2O$), black iron oxide ($FeO\cdot Fe_2O_3$)

No specific long-term feeding studies on red iron oxide (Fe_2O_3), yellow iron oxide ($FeO(OH)\cdot H2O$), or black iron oxide ($FeO\cdot Fe_2O_3$) were available.

3.2.5. Reproductive and developmental toxicity

Unspecified iron oxide

The JECFA evaluation on iron (1983) reported that 'an eight-generation reproduction study was carried out in Wistar rats. Dog food containing 570 mg of iron/lb as iron oxide was fed continuously. Rats ate an estimated 25 mg of iron/day, assuming 20 g/day of dog food consumption. No signs of toxicity were evident; reproduction performance was superior to expected values (Carnation Co., 1967)'.

The same JECFA evaluation on iron (1983) referred to another unpublished study (Kellog Co., 1968), in which 10 male and 3 female mink were fed 0.75 % iron oxide (unspecified compound) in their diet. Reproduction, whelping and lactation were observed to be similar to those of controls. Six male and four female pups then continued on the iron oxide diet until pelting (165 days), when acute nephrosis and hepatosis were observed. Fur quality and growth were normal.

The Panel noted that these studies were requested to the FDA but were not available, and therefore could not be evaluated.

The Panel also noted that in its evaluation on iron oxide, JECFA (1980) reported that 'rats consuming more than 50 mg/kg bw iron oxide for 8 generations showed no adverse effects on reproduction' (no additional information provided). Although not explicitly stated, the Panel assumed that the ADI of

[19] http://www.who.int/foodsafety/chem/jecfa/en/tox_guidelines.pdf

0.5 mg/kg bw/day established by JECFA (1980) could be derived from the Carnation Co. (1967) report by applying an uncertainty factor of 100 to the value of 50 mg/kg bw/day.

3.2.6. Allergenicity, hypersensitivity, intolerance

IUCLID dataset documents (EC, 2000a, b), reported both red iron oxide (Fe_2O_3) and black iron oxide ($FeO{\cdot}Fe_2O_3$) to be negative in the optimised sensitisation test method of Maurer (1979) using guinea pigs.

3.2.7. Other studies

3.2.7.1. *In vitro* studies with black iron oxide ($FeO{\cdot}Fe_2O_3$)

Elias et al. (1995) reported that the iron-containing mineral magnetite (black iron oxide, $FeO{\cdot}Fe_2O_3$) induced morphological transformations in SHE cells; the LC_{50} was approximately 80 µg/mL. However, according to the authors, when compared to nemalite (a fibrous iron-containing mineral), magnetite was 18-fold less potent in inducing the same transformation frequency.

Because of their use in magnetically-assisted haemodialysis, a new therapeutic application of iron oxide nanoparticles for the treatment of end-stage renal disease, Stamopoulos et al. (2010) evaluated the biocompatibility of black iron oxide ($FeO{\cdot}Fe_2O_3$) nanoparticles with red blood cells, white blood cells and platelets from a human donor. Optical microscopy and atomic force microscopy were employed for the morphological examination of blood cells that were matured in the presence of black iron oxide nanoparticles by incubation for up to 120 minutes at 20 °C. There was no noticeable interference between red blood cells, white blood cells and platelets with nanoparticles.

The interaction of Tween 80-coated superparamagnetic black iron oxide nanoparticles ($FeO{\cdot}Fe_2O_3$) of mean diameter 30 nm and murine macrophage (J774) cells was investigated to evaluate the dose and time-dependent toxic potential, as well as the role of oxidative stress as a mechanism for toxicity (Naqvi et al., 2010). An MTT assay showed more than 95 % viability of cells at lower concentrations (25–200 µg/mL) and up to 3 hours of exposure. At higher concentrations (300–500 µg/mL) and prolonged exposure (6 hours), the viability was reduced to 55–65 %. Necrosis/apoptosis assays revealed loss of the majority of the cells by apoptosis. Exposure to higher concentrations of nanoparticles resulted in enhanced ROS generation, leading to cell injury and death. The cell membrane injury induced by nanoparticles, studied using the lactate dehydrogenase assay, showed both concentration- and time-dependent damage. The authors concluded from this study that use of a low optimum concentration of super-paramagnetic black iron oxide nanoparticles is important for avoidance of oxidative stress-induced cell injury and death.

Ying and Hwang (2010) investigated the role of particle size and surface coating of iron oxide nanoparticles (presumably black iron oxide, $FeO{\cdot}Fe_2O_3$) on the cytotoxicity of A3 human T lymphocytes. Two different sizes (10 and 50 nm) and two different surface coatings (amine and carboxyl groups) of iron oxide nanoparticles were tested in a fluorescein diacetate assay and WST-1 assay. The 50 nm iron oxide nanoparticles were more toxic than those of 10 nm in the fluorescein diacetate assay after incubation of 1 or 24 hours. However, the results of both the 24-hour fluorescein diacetate and WST-1 assays using a complete growth medium indicated that the iron oxide nanoparticles of the smaller size are more toxic than those of the larger size.

3.2.7.2. Human studies

Hereditary haemochromatosis is an autosomal recessive disorder characterised by increased iron absorption (see reviews by Whittington and Kowdley, 2002; Crownover and Covey, 2013). The disease is, in most cases, due to a mutation of the HFE gene (C82Y), which codes for hepcidin, the primary iron regulatory hormone. The prevalence of the mutation in homozygous form ranges from 1 in 150 to 1 in 250 persons, and in heterozygous form is about 1 in 10 persons. However, most homozygotes are asymptomatic and only 10 % (1 in 2 500) exhibit organ toxicity or clinical manifestations (arthralgias, osteoporosis, cirrhosis, hepatocellular cancer, cardiomyopathy, diabetes

mellitus) of iron overload. Hereditary haemochromatosis is treated by phlebotomy to remove excess body stores of iron.

Dietary modifications are generally not needed, although patients with hereditary haemochromatosis should avoid iron and vitamin C supplements (Crownover and Covey, 2013). However, Hunt and Zeng (2004) studied iron absorption in HFE heterozygotes and found no higher iron absorption in this group compared to controls. The Panel is not aware of any study on the impact of iron as a food additive on the iron status of patients with hereditary haemochromatosis.

4. Discussion

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 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.

Iron oxides and hydroxides are a group of inorganic pigments collectively authorised for use as food additives (E 172) in the EU and previously evaluated by the SCF in 1975 and JECFA in 1974, 1975, 1978, 1980 and 2000 (JECFA, 1974, 1975, 1978, 1980, 2000).

Specifications for iron oxides and hydroxides have been defined in the EU legislation (Commission Regulation (EU) No 231/2012) and by JECFA (JECFA, 2008).

In the EC specifications for iron oxides and hydroxides (E 172) (Commission Regulation (EU) No 231/2012), three different oxides are listed:

- Yellow iron oxide: hydrated ferric oxide, hydrated iron (III) oxide, $FeO(OH)\cdot H_2O$, EINECS number 257-098-5, CAS number 51274-00-1,
- Red iron oxide: anhydrous ferric oxide, anhydrous iron (III) oxide, Fe_2O_3, EINECS number 215-168-2, CAS number 1309-37-1,
- Black iron oxide: ferroso ferric oxide, iron (II, III) oxide, $FeO\cdot Fe_2O_3$, EINECS number 235-442-5, CAS number 1317-61-9.

Brown Iron Oxide has been included in this assessment for completeness, due to its importance as a commercial blend: its colour shades are obtained by mixing different amounts of the aforementioned powdered principles. The Panel considered that only material with brown shades obtained by blending of the iron oxides and hydroxides evaluated in this Opinion would be covered by the present assessment.

As these iron oxides and hydroxides have different physical and chemical properties and they can be used separately, the Panel recommended that a clear differentiation (e.g. by adding a, b, c to the E number) be made between the different iron oxides and hydroxides that are currently all included under E 172.

According to the data previously submitted by industry (Rockwood, 2013a), the average particle sizes of iron oxide particles were 1 677, 318 and 957 nm for yellow iron oxide (FeO(OH)), red iron oxide (Fe_2O_3) and black iron oxide (Fe_3O_4), respectively. The Panel noted that the method used by industry for measuring the particle size of iron oxides (Rockwood, 2013a) cannot exclude the presence of particles with one or more dimensions below 100 nm.

More recently, TEM analyses were carried out on few E 172 products (Huntsman, 2015). Particle size distributions were found to vary in relation to the chemistry of the product, so that the distributions of primary particle sizes changed from FeO(OH) to Fe_2O_3 to Fe_3O_4. In all cases, particles that showed at least one dimension in the nanosize range were detected. The Panel had previously noted that,

according to the EFSA Guidance document, two different methods should be used to examine the particle size distribution (EFSA Scientific Committee, 2011).

In general, the Panel noted that the manufacturing process of powdered or particulate food additives results in material with a range of sizes. While the mean or median size of the particles is generally significantly greater than 100 nm, a small fraction will always be, and has been, with at least one dimension below 100 nm. The material used for toxicological testing would have contained this nano fraction. The test requirements stipulated in current EFSA guidance documents and EC guidelines for the intended use in the food/feed area apply in principle to unintended nano forms as well as to engineered nano material (ENM).

Therefore, the Panel considered that, in principle, for a specific food additive containing a fraction of particles with at least one dimension below 100 nm, adequately conducted toxicity tests should be able to detect hazards associated with this food additive including its nanoparticulate fraction. The Panel considered that for the re-evaluation of food additives this procedure would be sufficient for evaluating constituent nanoform fraction in accordance with the recommendation of the EFSA Nano Network in 2014.

Because of their importance in toxicokinetics and toxicological effects, the Panel considered that the particle size and particle size distribution should be included in the specifications of iron oxides and hydroxides. This should be performed by using appropriate methodologies as presented in the EFSA Guidance document (EFSA Scientific Committee, 2011).

The Panel noted that iron oxides and hydroxides are not authorised to be used as aluminium lakes for colouring purposes (Commission Regulation (EU) No 231/2012).

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 5–50 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 toxicity 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 1000 mg/kg bw/day, the highest dose tested.

No subchronic toxicity studies by oral administration of microsized yellow iron oxide (FeO(OH)), red iron oxide (Fe_2O_3) or black iron oxide ($FeO{\cdot}Fe_2O_3$) were available. The subchronic toxicity of red iron oxide (Fe_2O_3) 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 Fe showed no dose-associated response in comparison with 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 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 a similar range of daily doses, adverse effects were observed in rats subacutely treated (28 days) with red iron oxide nanoparticles, while 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 subacute 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 (Fe_2O_3) and black ($FeO{\cdot}Fe_2O_3$) 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 1 to 9 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 Cat Care Center (1968), no adverse effects were reported in cats maintained on diets containing 1 900 mg/kg diet (475 mg/kg bw/day) of iron from iron oxide (equivalent to 0.27 % iron oxide) for periods of 2–9 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 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 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.

Regarding Brown Iron Oxide, the E 172 brown shade is mentioned in Commission Regulation (EU) No 231/2012, although the blend itself is nominally not listed, nor further characterised. The Panel noted that specifications and a reliable toxicological database on yellow, red and black iron oxides are needed in order to assess its safety when used as a food additive.

Exposure assessment of iron oxides and hydroxides (E 172) was carried out by the ANS Panel based on (1) maximum reported use levels (defined as the *maximum level exposure assessment scenario*) and (2) reported use levels (defined as the *refined exposure assessment scenario*) as provided to EFSA by industry. The Panel considered that the refined exposure assessment approach results in more realistic long-term exposure estimates because of the underlying assumptions and the concentration data used. The Panel noted that due to limited information becoming available on the type of iron oxides and hydroxides (yellow, red or black) used in the authorised food categories, the exposure estimates for E 172 were based on MLs/reported use levels irrespectively of the type of iron oxide.

The Panel noted that only 10 out of the 49 food categories in which iron oxides and hydroxides (E 172) are authorised were taken into account in the present exposure estimates and therefore, that overall this would result in an underestimation of the actual exposure to iron oxides and hydroxides (E 172) as food additives in European countries.

The Panel also noted that the refined exposure estimates will not cover future changes in the level of use of any type of iron oxides and hydroxides (E 172).

Using the *maximum level exposure assessment scenario*, mean exposure to E 172 from its use as a food additive ranged from 0.1 mg/kg bw/day for infants to 10.5 mg/kg bw/day for toddlers, while the high exposure using this scenario ranged from 0.2 mg/kg bw/day for infants to 26.9 mg/kg bw/day for toddlers. Using the *refined brand-loyal assessment exposure scenario*, mean exposure to E 172 from its use as a food additive ranged from 0.1 mg/kg bw/day for infants to 8.9 mg/kg bw/day for toddlers. The high exposure to E 172 using this scenario ranged from 0.2 mg/kg bw/day for infants to 23.1 mg/kg bw/day in toddlers. Using the *refined non-brand-loyal assessment exposure scenario*, mean exposure to E 172 from its use as food additive ranged from 0.03 mg/kg bw/day for infants to 3.7 mg/kg bw/day for toddlers. The high exposure to E 172 from its use as food additive using this scenario ranged from 0.1 mg/kg bw/day for infants to 9.5 mg/kg bw/day for toddlers. Overall, the lowest exposure to iron oxides and hydroxides (E 172) was estimated for infants, while the highest

exposure was calculated for toddlers, in all scenarios. The food categories that, at the individual level, had the highest contribution to the total individual exposure to iron oxides and hydroxides (E 172) were fine bakery wares and desserts, excluding products covered in categories 1, 3 and 4.

CONCLUSIONS

The 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.

RECOMMENDATIONS

The Panel noted that for the food additive iron oxides and hydroxides (E 172), the term 'iron oxides' applies sometimes either to iron oxides or iron hydroxides and therefore grouping them together under a single E number is confusing. As these compounds have different physical and chemical properties and they can be used separately, the Panel recommended that a clear differentiation (e.g. by adding a, b, c to the E number) should be made between the different iron oxides and hydroxides that are currently all included under E 172. Furthermore, the Panel noted that concentration data on yellow iron oxide, red iron oxide and black iron oxide alone would be needed for the calculation of exposure estimates for each of the three single iron oxides.

Because of the potential importance of nanoparticles in toxicokinetics and toxicological effects, the Panel considered that the particle size and particle size distribution should be included in the specifications of iron oxides and hydroxides (E 172).

The Panel considered that the maximum limits for certain toxic elements (cadmium, arsenic, lead and mercury) present as impurities in the EC specification for iron oxides and hydroxides (E 172) should be revised in order to ensure that iron oxides and hydroxides (E 172) as food additives will not be a significant source of exposure to these toxic elements in foods. It is also recommended that the limit specified in the EC specifications for chromium should be for the presence of chromium(III) and absence of chromium(VI).

Considering the differences in physical-chemical characteristics of the particulate material (redox states, particle size) between the different iron oxides, the Panel recommended that additional data should be provided on these compounds.

The Panel recommended that the minimum, Tier 1 testing according to the EFSA guidance (2012), should be conducted for the material as marketed as the food additive (E 172):

- red iron oxide: *in vivo* genotoxicity at the site of contact (gastrointestinal tract) and subchronic toxicity,
- yellow iron oxide: a complete set of genotoxicity studies and subchronic toxicity,
- black iron oxide: ADME, *in vivo* genotoxicity and subchronic toxicity.

DOCUMENTATION PROVIDED TO EFSA

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APPENDICES

APPENDIX A: SUMMARY OF THE REPORTED USE LEVELS (MG/KG OR MG/L AS APPROPRIATE) OF IRON OXIDES AND HYDROXIDES (E 172) PROVIDED BY INDUSTRY

FCS category no.	FCS food category	MPL	Restrictions	Reported use levels			Information provided by
				n	Typical mean	Highest maximum level	
03	Edible ices	QS		1	244.0	244.0	FDE
05.2	Other confectionery including breath refreshening microsweets	QS		1	85.0	700.0	FDE
05.3	Chewing gum	QS		1	570.0	1 936.0	ICGA
05.4	Decorations, coatings and fillings, except fruit based fillings covered by category 4.2.4	QS		3	3751.9	10 000.0	FDE
07.2	Fine bakery wares	QS		2	590.0	2 000.0	FDE
08.3.3	Casings and coatings and decorations for meat	QS	Except edible external coating of pasturmas	2	25.0	50.0	FDE
14.1.4	Flavoured drinks	QS	Excluding chocolate milk; malt products	1 NP	15.0	15.0	FDE
15.1	Potato-, cereal-, flour- or starch-based snacks	QS		1	176.4	176.4	FDE
16	Desserts excluding products covered in categories 1, 3 and 4	QS		1	750.0	2 000.0	FDE
17.1	Food supplements supplied in a solid form including capsules and tablets and similar forms excluding chewable forms	QS		1	1,92	2.88	Capsugel (for empty capsules from 1 EU country)
				11	1 715	5 000.0	AESGP (data from 3 different European countries and 2 data from EU
17.3	Food supplements supplied in a syrup-type or chewable form	QS		4	2 420.0	3 500.0	AESGP (on chewing tablet); from 4 different European countries (not representative of all EU)

NP : niche products

APPENDIX B: CONCENTRATION LEVELS OF IRON OXIDES AND HYDROXIDES (E 172) USED IN THE REFINED EXPOSURE SCENARIOS (MG/KG OR MG/ML AS APPROPRIATE)

FCS category no.	FCS food category	Restrictions/exceptions	MPL	Concentration levels		Comments
				Mean	Maximum	
01.4	Flavoured fermented milk products including heat treated products		QS	-	-	Not taken into account (no usage data available)
01.5	Dehydrated milk as defined by Directive 2001/114/EC	Except unflavoured products	QS	-	-	Not taken into account (no usage data available)
01.6.3	Other creams	Only flavoured creams	QS	-	-	Not taken into account (no usage data available)
01.7.1	Unripened cheese excluding products falling in category 16	Only flavoured unripened cheese	QS	-	-	Not taken into account (no usage data available)
01.7.3	Edible cheese rind		QS	-	-	Not taken into account (no usage data available/no corresponding FoodEx code)
01.7.4	Whey cheese		QS	-	-	Not taken into account (no usage data available)
01.7.5	Processed cheese	Only flavoured processed cheese	QS	-	-	Not taken into account (no usage data available)
01.7.6	Cheese products (excluding products falling in category 16	Only flavoured unripened products	QS	-	-	Not taken into account (no usage data available/no corresponding FoodEx code)
01.8	Dairy analogues, including beverage whiteners		QS	-	-	Not taken into account (no usage data available)
03	Edible ices		QS	244	244	
04.1.1	Entire fresh fruit and vegetables	Only as a contrast enhancer for marking citrus fruit, melons and pomegranates in order to: repeat all or some of the mandatory information particulars required by the Union legislation and/or national law, and/or provide on a voluntary basis brand name, production method, PLU-code, QR-code and/or barcode	6	-	-	Not taken into account (no usage data available)

FCS category no.	FCS food category	Restrictions/exceptions	MPL	Concentration levels		Comments
				Mean	Maximum	
04.2.4.1	Fruit and vegetable preparations excluding compote	Only mostarda di frutta	QS	-	-	Not taken into account (no usage data available/no corresponding FoodEx code)
04.2.4.1	Fruit and vegetable preparations excluding compote	Only seaweed based fish roe analogues	QS	-	-	Not taken into account (no usage data available/no corresponding FoodEx code)
04.2.5.3	Other similar fruit or vegetable spreads	Except *crème de pruneaux*	QS	-	-	Not taken into account (no usage data available)
05.2	Other confectionery including breath freshening microsweets		QS	85	700	
05.3	Chewing gum		QS	570	1 936	
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4		QS	-	-	Not taken into account (no corresponding FoodEx code)
06.3	Breakfast cereals	Only breakfast cereals other than extruded, puffed and/or fruit-flavoured breakfast cereals	QS	-	-	Not taken into account (no usage data available)
06.5	Noodles		QS	-	-	Not taken into account (no usage data available)
06.6	Batters		QS	-	-	Not taken into account (no usage data available/no corresponding FoodEx code)
06.7	Pre-cooked or processed cereals		QS	-	-	Not taken into account (no usage data available/ no corresponding FoodEx code)
07.2	Fine bakery wares		QS	590	2 000	
08.3.3	Casings and coatings and decorations for meat	Except edible external coating of *pasturmas*	QS	-	-	Not taken into account (no corresponding FoodEx code)
09.2	Processed fish and fishery products including molluscs and crustaceans	Only surimi and similar products and salmon substitute	QS	-	-	Not taken into account (no usage data available)
09.2	Processed fish and fishery products including molluscs and crustaceans	Only fish paste and crustacean paste	QS	-	-	Not taken into account (no usage data available)
09.2	Processed fish and fishery products including molluscs and crustaceans	Only smoked fish	QS	-	-	Not taken into account (no usage data available)

FCS category no.	FCS food category	Restrictions/exceptions	MPL	Concentration levels		Comments
				Mean	Maximum	
09.3	Fish roe	Except Sturgeons' eggs (Caviar)	QS	-	-	Not taken into account (no usage data available)
12.2.2	Seasonings and condiments	Only seasonings, for example curry powder, tandoori	QS	-	-	Not taken into account (no usage data available)
12.4	Mustard		QS	-	-	Not taken into account (no usage data available)
12.5	Soups and broths		QS	-	-	Not taken into account (no usage data available)
12.6	Sauces	Excluding tomato-based sauces	QS	-	-	Not taken into account (no usage data available)
12.7	Salads and savoury-based sandwich spreads		QS	-	-	Not taken into account (no usage data available)
12.9	Protein products, excluding products covered in category 1.8		QS	-	-	Not taken into account (no usage data available)
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)		QS	-	-	Not taken into account (no usage data available)
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)		QS	-	-	Not taken into account (no usage data available)
13.4	Foods suitable for people intolerant to gluten as defined by Regulation (EC) No 41/2009		QS	-	-	Not taken into account (no usage data available)
14.1.4	Flavoured drinks	Excluding chocolate milk and malt products	QS	15	15	
14.2.3	Cider and perry	Excluding cidre bouché	QS	-	-	Not taken into account (no usage data available)
14.2.4	Fruit wine and made wine	Excluding *wino owocowe markowe*	QS	-	-	Not taken into account (no usage data available/no corresponding FoodEx code)
14.2.5	Mead		QS	-	-	Not taken into account (no usage data available/no corresponding FoodEx code)

FCS category no.	FCS food category	Restrictions/exceptions	MPL	Concentration levels		Comments
				Mean	Maximum	
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008	Except: spirit drinks as defined in Article 5(1) and sales denominations listed in Annex II, paragraphs 1-14 of Regulation (EC) No 110/2008 and spirits (preceded by the name of the fruit) obtained by maceration and distillation, Geist (with the name of the fruit or the raw material used), London Gin, Sambuca, Maraschino, Marrasquino or Maraskino and Mistrà	QS	-	-	Not taken into account (no usage data available)
14.2.7.3	Aromatised wine-product cocktails		QS	-	-	Not taken into account (no usage data available/no corresponding FoodEx code)
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15 % alcohol		QS	-	-	Not taken into account (no usage data available)
15.1	Potato-, cereal-, flour- or starch-based snacks		QS	176	176	
15.2	Processed nuts		QS	-	-	Not taken into account (no usage data available)
16	Desserts excluding products covered in categories 1, 3 and 4		QS	750	2 000	
17.1	Food supplements supplied in a solid form including capsules and tablets and similar forms, excluding chewable forms		QS	1 900	5 000	
17.2	Food supplements supplied in a liquid form		QS			
17.3	Food supplements supplied in a syrup-type or chewable form		QS			

APPENDIX C: SUMMARY OF TOTAL ESTIMATED EXPOSURE TO IRON OXIDES AND HYDROXIDES (E 172) FROM THEIR USE AS FOOD ADDITIVES FOR THE MAXIMUM LEVEL EXPOSURE SCENARIO AND THE REFINED EXPOSURE ASSESSMENT SCENARIOS PER POPULATION GROUP AND SURVEY: MEAN AND HIGH LEVEL (MG/KG BW/DAY)

	Number of subjects	Maximum level scenario		Brand-loyal scenario		Non-brand-loyal scenario	
		Mean	High level	Mean	High level	Mean	High level
Infants							
Bulgaria (NUTRICHILD)	859	1.7	8.9	1.7	8.9	0.5	2.6
Germany (VELS)	159	1.6	8.0	1.5	7.6	0.6	2.5
Denmark (IAT 2006 07)	826	1.1	5.8	1.1	5.5	0.4	2.2
Finland (DIPP 2001 2009)	500	0.1	0.2	0.1	0.2	0.0	0.1
United Kingdom (DNSIYC 2011)	1 366	2.2	11.7	2.0	10.5	0.8	4.2
Italy (INRAN SCAI 2005 06)	16	0.1	-	0.1	-	0.0	-
Toddlers							
Belgium (Regional Flanders)	36	7.2	-	6.3	-	2.7	-
Bulgaria (NUTRICHILD)	428	6.4	17.0	6.4	16.9	2.0	5.2
Germany (VELS)	348	7.1	18.7	6.1	14.8	2.5	6.5
Denmark (IAT 2006 07)	917	1.9	6.5	1.6	5.6	0.7	2.3
Spain (enKid)	17	5.6	-	4.7	-	1.9	-
Finland (DIPP 2001 2009)	500	0.4	1.7	0.4	1.6	0.2	0.7
United Kingdom (NDNS-RollingProgrammeYears1-3)	185	5.7	16.8	5.0	13.9	2.0	6.0
United Kingdom (DNSIYC 2011)	1 314	4.7	14.7	4.2	13.5	1.7	5.4
Italy (INRAN SCAI 2005 06)	36	3.3	-	3.2	-	1.0	-
Netherlands (VCP kids)	322	10.5	26.9	8.9	23.1	3.7	9.5
Children							
Austria (ASNS Children)	128	5.0	12.8	4.9	12.4	1.7	3.8
Belgium (Regional Flanders)	625	7.0	17.1	6.1	14.4	2.5	6.0
Bulgaria (NUTRICHILD)	433	7.3	18.6	7.2	18.6	2.3	5.8
Czech Republic (SISP04)	389	5.9	14.9	5.4	13.3	2.0	4.8
Germany (EsKiMo)	835	3.3	9.2	3.0	8.1	1.2	3.1
Germany (VELS)	293	7.3	16.5	6.2	14.0	2.6	5.8
Denmark (DANSDA 2005-08)	298	1.4	4.0	1.2	3.4	0.6	1.6
Spain (enKid)	156	4.8	14.5	4.4	13.0	1.6	4.7
Spain (NUT INK05)	399	4.1	12.0	3.8	10.4	1.4	4.1
Finland (DIPP 2001 2009)	750	1.6	5.2	1.5	5.0	0.5	1.3
France (INCA2)	482	9.2	19.4	7.8	15.8	3.0	6.3
United Kingdom (NDNS-RollingProgrammeYears1-3)	651	5.2	12.7	4.5	10.6	1.8	4.3
Greece (Regional Crete)	838	5.5	13.8	5.4	13.0	1.8	4.3
Italy (INRAN SCAI 2005 06)	193	4.0	10.4	4.0	10.1	1.3	3.3
Latvia (EFSA TEST)	187	4.8	13.6	4.6	13.6	1.6	5.0

	Number of subjects	Maximum level scenario		Brand-loyal scenario		Non-brand-loyal scenario	
		Mean	High level	Mean	High level	Mean	High level
Netherlands (VCP kids)	957	8.8	21.9	7.5	19.2	3.1	7.6
Netherlands (VCPBasis AVL2007 2010)	447	7.4	17.4	6.2	14.9	2.6	6.1
Sweden (NFA)	1 473	5.2	13.0	4.8	12.2	1.9	4.4
Adolescents							
Austria (ASNS Children)	237	2.6	7.8	2.5	7.7	0.9	2.4
Belgium (Diet National 2004)	576	2.7	7.7	2.6	6.8	0.9	2.5
Cyprus (Childhealth)	303	1.4	4.3	1.4	3.9	0.5	1.3
Czech Republic (SISP04)	298	4.0	10.6	3.8	9.9	1.3	3.4
Germany (National Nutrition Survey II)	1 011	2.2	8.3	2.0	7.5	0.7	2.7
Germany (EsKiMo)	393	2.2	6.2	2.0	5.3	0.8	2.0
Denmark (DANSDA 2005-08)	377	0.7	2.2	0.6	1.7	0.3	0.8
Spain (AESAN FIAB)	86	2.5	6.6	2.3	6.4	0.8	2.0
Spain (enKid)	209	3.1	8.4	2.8	8.3	1.0	2.8
Spain (NUT INK05)	651	2.4	6.8	2.3	6.5	0.8	2.2
Finland (NWSSP07 08)	306	1.0	3.5	0.9	3.3	0.2	0.7
France (INCA2)	973	4.5	10.7	3.9	9.5	1.5	3.4
United Kingdom (NDNS-RollingProgrammeYears1-3)	666	2.3	6.8	2.1	5.7	0.8	2.3
Italy (INRAN SCAI 2005 06)	247	2.1	6.4	2.1	6.4	0.7	2.2
Latvia (EFSA TEST)	453	3.0	9.2	2.9	8.9	1.0	3.3
Netherlands (VCPBasis AVL2007 2010)	1 142	4.4	11.3	3.8	9.3	1.6	4.0
Sweden (NFA)	1 018	2.8	8.1	2.6	7.6	1.0	2.6
Adults							
Austria (ASNS Adults)	308	2.4	7.5	2.3	7.3	0.8	2.3
Belgium (Diet National 2004)	1 292	1.8	5.8	1.7	5.6	0.6	1.9
Czech Republic (SISP04)	1 666	1.8	5.9	1.8	5.8	0.6	1.8
Germany (National Nutrition Survey II)	10 419	2.0	6.5	1.9	6.1	0.6	2.0
Denmark (DANSDA 2005-08)	1 739	0.5	1.4	0.4	1.2	0.2	0.5
Spain (AESAN)	410	1.4	4.8	1.4	4.7	0.5	1.5
Spain (AESAN FIAB)	981	1.7	4.9	1.6	4.7	0.5	1.5
Finland (FINDIET2012)	1 295	1.8	5.5	1.6	4.8	0.6	1.7
France (INCA2)	2 276	2.3	6.3	2.1	5.5	0.8	2.0
United Kingdom (NDNS-RollingProgrammeYears1-3)	1 266	1.3	3.8	1.2	3.5	0.4	1.3
Hungary (National Repr Surv)	1 074	0.4	2.0	0.4	2.0	0.1	0.6
Ireland (NANS 2012)	1 274	1.6	4.5	1.5	4.3	0.5	1.4
Italy (INRAN SCAI 2005 06)	2 313	1.0	3.2	1.0	3.1	0.3	1.0
Latvia (EFSA TEST)	1 271	1.6	5.5	1.5	5.2	0.5	1.7
Netherlands (VCPBasis AVL2007 2010)	2 057	2.4	6.9	2.1	5.8	0.8	2.4
Romania (Dieta Pilot Adults)	1 254	0.3	1.3	0.3	1.2	0.1	0.5
Sweden (Riksmaten 2010)	1 430	1.6	5.1	1.4	4.7	0.5	1.6

	Number of subjects	Maximum level scenario		Brand-loyal scenario		Non-brand-loyal scenario	
		Mean	High level	Mean	High level	Mean	High level
The elderly							
Austria (ASNS Adults)	92	2.3	6.8	2.2	6.4	0.7	2.1
Belgium (Diet National 2004)	1 215	1.7	5.6	1.6	5.0	0.6	1.9
Germany (National Nutrition Survey II)	2 496	2.0	6.4	1.9	6.0	0.6	2.0
Denmark (DANSDA 2005-08)	286	0.4	1.3	0.4	1.2	0.1	0.4
Finland (FINDIET2012)	413	1.7	5.5	1.6	5.0	0.5	1.6
France (INCA2)	348	1.7	5.3	1.6	4.6	0.6	1.6
United Kingdom (NDNS-RollingProgrammeYears1-3)	305	1.9	4.9	1.7	4.4	0.6	1.7
Hungary (National Repr Surv)	286	0.5	2.6	0.5	2.6	0.2	0.8
Ireland (NANS 2012)	226	1.9	5.2	1.7	4.9	0.6	1.7
Italy (INRAN SCAI 2005 06)	518	0.8	2.6	0.7	2.5	0.2	0.8
Netherlands (VCPBasis AVL2007 2010)	173	2.3	6.7	2.0	5.4	0.8	2.3
Netherlands (VCP-Elderly)	739	2.3	6.3	2.0	5.1	0.8	2.2
Romania (Dieta Pilot Adults)	128	0.3	1.0	0.3	1.0	0.1	0.4
Sweden (Riksmaten 2010)	367	1.9	5.3	1.8	4.7	0.6	1.6

ABBREVIATIONS

AAS	atomic absorption spectrometry
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
AESGP	Association of the European Self-Medication Industry
AFC	EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
ANS Panel	EFSA Panel on Food Additives and Nutrient Sources added to Food
AOAC	Association of Official Analytical Chemists
B[a]P	benzo(a)pyrene
BHA	butylated hydroxyanisole
BNMN	Binucleate micronucleated leukocytes
CBPI	cytokinesis block proliferation index
CFR	Code of Federal Regulations
CI	Colour Index
CIAA	Confederation of the Food and Drink Industries of the EU
CONTAM Panel	EFSA Panel on Contaminants in the Food Chain
DLS	dynamic light scattering
DMSO	dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DR	diffuse reflectance
EC	European Commission
ECHA	European Chemicals Agency
EDXRF	energy-dispersive X-ray fluorescence spectrometry
EFSA	European Food Safety Authority
ENM	engineered nanomaterials
EU	European Union
FCS	Food Categorisation System
FDA	Food and Drug Administration
FDE	FoodDrinkEurope
FPG	formamidopyrimidine DNA glycosylase
FSANZ	Food Standards Australia New Zealand
GMP	Good Manufacturing Practice
IARC	International Agency for Research in Cancer
ICGA	International Chewing Gum Association
ICP-AES	inductively coupled plasma-atomic emission spectroscopy
ICP-MS	inductively coupled plasma-mass spectrometry among

ICP-OES	inductively coupled plasma optical emission spectrometry
INS	International Numbering System
IUCLID	International Uniform Chemical Information Database
JECFA	Joint FAO/WHO Expert Committee of Food Additives
LD_{50}	lethal dose, 50 %, i.e. dose that causes death among 50 % of treated animals
LDT	laser diffraction technique
MOE	Margin of Exposure
MPL	maximum permitted levels
NAC	*N*-acetyl-cysteine
NOAEL	no-observed-adverse effect
OECD	Organisation for Economic Co-operation and Development
OTM	Olive tail moment
ROS	reactive oxygen species
SCF	Scientific Committee on Food
SHE	Syrian hamster embryo
TEM	transmission electron microscopy
TI	Tail Intensity
TG	Test Guideline
UDS	unscheduled DNA synthesis