SCIENTIFIC OPINION

Scientific Opinion on the use of ferric sodium EDTA as a source of iron added for nutritional purposes to foods for the general population (including food supplements) and to foods for particular nutritional uses[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 on ferric sodium EDTA added for nutritional purposes to foods for the general population (including food supplements) and to PARNUTS. Iron from ferric sodium EDTA is 2 to 3 times more bioavailable than from other mineral sources. In PARNUTS and food supplements ferric sodium EDTA would provide up to 22.3 mg iron/day (children) and 11.1 mg/day (adults). In fortified foods the proposed uses would result in an additional intake of iron of 2.2 mg/day (children) and 4.8 mg/day (male adults) on average and of 4.8 mg/day and 11.3/day respectively at the 95th percentile. The combined exposure to EDTA in the proposed applications amounts to 8.6 mg/kg bw/day (children) and 4.2 mg/kg bw/day (adults) on average and to 9.5 mg/kg bw/day and 4.8 mg/kg bw/day respectively at the 95th percentile. From two 90-day rat studies the Panel derives a NOAEL of 250 mg ferric sodium EDTA/kg bw/day. The Panel considers that from the information available there is no safety concern with respect to genotoxicity of ferric sodium EDTA as a source of iron added for nutritional purposes to foodstuffs. Chronic toxicity or carcinogenicity studies have not been provided. However, from relevant studies with other EDTA salts the Panel concludes that ferric sodium EDTA does not raise concern with respect to carcinogenicity. There is no concern on a carcinogenic potential of EDTA. No ADI for EDTA has been established. However, from the ADI of 2.5 mg/kg bw/day established for calcium disodium EDTA a value of 1.9 mg EDTA/kg bw/day can be calculated. The Panel concludes that ferric sodium EDTA as a source of iron in food supplements, PARNUTS and fortified foods is of no safety concern at the proposed use levels as long as it does not lead to an exposure to EDTA above 1.9 mg EDTA/kg bw/day.

KEY WORDS

Ferric sodium EDTA, CAS No: 18154-32-0; ferric sodium EDTA, anhydrous CAS No: 15708-41-5; ferrate(1-), [(ethylenedinitrilo)tetraacetato]-, sodium; ferric ethylenediaminetetraacetic acid, sodium salt; ferric sodium edentate; iron monosodium EDTA; iron sodium ethylenediaminetetraacetate (1:1:1); sodium [(ethylenedinitrilo)tetraacetato]ferrate(III); sodium (N,N,N',N'-ethylenediaminetetraacetato)ferrate(1-); sodium ferric ethylenediaminetetraacetate; Sodium iron(III) ethylenediaminetetraacetate; sodium [(ethylenedinitrilo)tetraacetatato]ferrate(III).

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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 on ferric sodium EDTA added for nutritional purposes to foods for the general population (including food supplements) and to foods for particular nutritional uses (PARNUTS).

The present opinion deals only with the safety of ferric sodium EDTA as a source of iron and with the bioavailability of iron from this source. The safety of iron itself, in terms of the amounts that may be consumed, is outside the remit of this Panel.

Information has been provided on the bioavailability of iron from ferric sodium EDTA based on iron fortification studies in humans. From these studies the Panel deduced that iron is liberated from the complex and that it is bioavailable. The studies further show that iron in the form of ferric sodium EDTA is 2 to 3 times more bioavailable than iron in the form of ferrous sulfate and that it is efficiently incorporated into haemoglobin.

The Panel notes that the absorption of iron from ferric sodium EDTA is regulated physiologically by the body's iron status, in a manner similar to that for other iron compounds and that dietary iron fortification with ferric sodium EDTA is not expected to result in iron overload in iron-repleted individuals.

The Panel also notes that studies have been conducted to investigate the effect of ferric sodium EDTA on the absorption and metabolism of other nutrients in food (i.e. zinc, copper, calcium, manganese, and magnesium) in animals (rat) and in humans (fortification studies) and that no influence has been observed.

The Panel notes that two 90-day studies with ferric sodium EDTA it the rat have been provided. From the data provided the Panel derives an overall NOAEL of 250 mg ferric sodium EDTA/kg bw/day.

From a 61-day feeding study in the rat a NOAEL of 84.3 mg/kg bw/day of ferric sodium EDTA (providing 11.2 mg iron/kg bw/day) could be derived. The Panel notes that based on the results of this study, the JECFA concluded in 2000, that administration of ferric sodium EDTA in the diet would not result in a greater uptake of iron once nutritional requirements for iron were met.

The Panel notes that *in vitro* mutagenicity assays conducted in S. *typhimurium* (7 strains) and E. *coli* (2 strains) were negative, while in an *in vitro* mouse lymphoma assay a weak positive response in the presence of moderate cytotoxicity was observed. However, similar results were observed with other iron compounds in this *in vitro* mouse lymphoma assay and the effects observed with sodium iron (III) EDTA could probably be attributed to iron rather than to EDTA. Additionally, an *in vivo* mouse micronucleus assay was negative. Furthermore, in an EU Risk Assessment Report on EDTA, it is concluded that "EDTA and its sodium salts have a low mutagenic potential at extremely high doses. On the basis of the various negative findings and the assumption of a threshold mode-of action for aneugens, it can be concluded that EDTA and its sodium salts are not mutagenic for humans." Thus, the Panel considers that from the information available there is no safety concern with respect to genotoxicity of ferric sodium EDTA as a source of iron added for nutritional purposes to foodstuffs.

No chronic toxicity or carcinogenicity studies have been conducted with ferric sodium EDTA; however, several studies have been conducted with other EDTA salts (e.g. trisodium EDTA, calcium disodium EDTA and disodium EDTA). Ferric sodium EDTA, like other EDTA-metal complexes, dissociates in the gut to a bioavailable form of iron and an EDTA salt; hence, toxicology studies of other EDTA salts are relevant when considering the safety of ferric sodium EDTA. From these studies it can be concluded that EDTA salts do not raise concern with respect to carcinogenicity.

From data on developmental studies conducted in the rat using similar EDTA salts, such as disodium EDTA, trisodium EDTA, tetrasodium EDTA, and calcium disodium EDTA, no compound-related mortality, reproductive, or teratogenic effects were reported.

From a developmental toxicity study on ferric sodium EDTA in the rat the Panel derived a NOAEL of 200 mg/kg bw/day.

Ferric sodium EDTA has been used in numerous field trials on iron fortification of foods in developing countries. From these studies it appears that no adverse effects were reported in humans subjected to long-term ferric sodium EDTA fortification trials.

The Panel notes that photodegradation of EDTA can give rise to the formation of formaldehyde. The EFSA AFC Panel examined formaldehyde when used as a preservative during the manufacture and preparation of food additives and concluded that there was no evidence indicating that formaldehyde is carcinogenic by the oral route. Therefore the Panel considers that the potential formation of formaldehyde as a degradation product of EDTA is not expected to pose a safety concern in humans under the proposed conditions of use of ferric sodium EDTA.

The petitioner indicates that ferric sodium EDTA will be used in PARNUTS to provide 22.3 mg iron/day for a 60 kg adult and 11.1 mg iron/day for a 30 kg child. To provide these levels of iron, equivalent values of ferric sodium EDTA of about 168 mg/day and 84 mg/day respectively will be needed.

In the case of food supplements, the petitioner did not indicate precise use levels, but states that these are similar to that of other forms of iron currently approved for use in food supplements. The petitioner expects the iron intake from the use of ferric sodium EDTA not to exceed 22.3 mg/day for a 60 kg adult or 11.1 mg/day for a 30 kg child. Equally to the use of ferric sodium EDTA as a source of iron in PARNUTS, to provide these levels of iron in the case of food supplements, equivalent values of ferric sodium EDTA of about 168 mg/day and 84 mg/day respectively will be needed.

The Panel notes that the EVM has stated that for guidance purposes only, a supplemental intake of approximately 17 mg iron/day (equivalent to 0.28 mg/kg bw/day for a 60 kg adult) would not be expected to produce adverse effects in the majority of people. An amount of 17 mg iron/day would be provided by 128.3 mg ferric sodium EDTA providing 89 mg EDTA, equivalent to about 1.5 mg EDTA/kg bw/day for an adult and 5.9 mg EDTA/kg bw/day for children weighing 15 kg.

Based on these intake values the Panel calculated the resulting intake of EDTA if all iron were to be provided by ferric sodium EDTA. In the case of PARNUTS the exposure to EDTA would amount to about 116 mg/day for adults and to about 58 mg/day for children. In the case of food supplements the exposure to EDTA would also amount to 116 mg/day for adults and to about 58 mg/kg bw/day for children. The Panel notes that in both cases the exposure to EDTA will be about 1.9 mg EDTA/kg bw/day for adults and 3.9 mg EDTA/kg bw/day for children weighing 15 kg.

In the case of food fortification, assuming the use levels of ferric sodium EDTA as provided by the petitioner, the total exposure to EDTA would be 11.3 mg/day for children (15 kg) and 24.6 mg/day for male adults on average, and 24.6 mg/day and 58.5 mg/day, respectively, at the 95th percentile. On a body weight basis, this is equivalent to 0.8 and 0.4 mg/kg bw/day at the average, and 1.7 and 1.0 mg/kg bw/day at the 95th percentile.

The Panel notes that no ADI for EDTA has been set but that the JECFA established an ADI for calcium disodium EDTA of 2.5 mg/kg bw/day, which can be calculated to amount to 1.9 mg EDTA/kg bw/day. Calcium disodium EDTA is the only currently approved EDTA derivative in the EU.

If assumed that ferric sodium EDTA as a source for iron would be consumed from all three sources (PARNUTS, fortified foods and supplements), the combined exposure to EDTA would be 8.6 mg/kg bw/day for children and 4.2 mg/kg bw/day for adults on average and to 9.5 mg/kg bw/day for children and 4.8 mg/kg bw/day for adults at the 95th percentile. This exceeds the value for EDTA of 1.9 mg/kg bw/day derived from the ADI established for calcium disodium EDTA. The Panel cannot estimate the probability that an individual is exposed to all products where ferric sodium EDTA is intended to be added as a source for iron although it considers this as rather unlikely.

The exposure to sodium resulting from ferric sodium EDTA intended to provide 22.3 mg iron/day (equivalent to 165 mg ferric sodium EDTA) would result in an additional exposure to 9 mg sodium/day. Compared to the typical dietary exposure to sodium in the average range of 4,500-11,000 mg/day across Europe, this additional exposure is considered to be irrelevant even under concurrent exposure to ferric sodium EDTA from different sources.

The Panel concludes that iron is bioavailable from ferric sodium EDTA and that the use of ferric sodium EDTA as a source of iron in food is of no safety concern as long as it does not lead to an exposure to EDTA above 1.9 mg EDTA/kg bw/day.

The Panel further concludes that ferric sodium EDTA as a source of iron at the proposed use level in fortified food for the general population would not be of safety concern.

The Panel notes that when ferric sodium EDTA is used in PARNUTS or food supplements at levels which provide 22.3 mg iron/day for an adult and 11.1 mg iron/day for a child, the corresponding exposure to EDTA would be 1.9 mg EDTA/kg bw/day for adults and 3.9 mg/kg bw/day for children.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The European Community legislation lists nutritional substances that may be used for nutritional purposes in certain categories of foods as sources of certain nutrients.

The Commission has received a request for the evaluation of ferric sodium EDTA added for nutritional purposes to food for the general population (including food supplements) and food for particular nutritional uses. The relevant Community legislative measure is:

- Commission Directive 2001/15/EC of 15 February 2001 on substances that may be added for specific nutritional purposes in foods for particular nutritional uses[4].
- Directive 2002/46/EC of the European Parliament and of the Council on the approximation of the laws of the Member States relating to food supplements[5].
- Regulation (EC) 1925/2006 on the addition of vitamins and minerals and of certain other substances to food[6].

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority to:

- carry out the additional assessment for ferric sodium EDTA as a source of iron added for nutritional purposes to foodstuffs, in the context of Regulation (EC) N° 258/97, and,
- provide a scientific opinion, based on its consideration of the safety and bioavailability of ferric sodium EDTA added for nutritional purposes in food for the general population (including food supplements) and foods for particular nutritional uses.

[4] OJ L 52, 22.2.2001, p. 19
[5] OJ L 183,12.7.2002, p. 51
[6] OJ L 404, 30.12.2006, p. 26

ASSESSMENT

1. Introduction

The present opinion deals with the safety of ferric sodium EDTA and with the bioavailability of the iron from this source. The safety of iron itself, in term of amounts that may be consumed, is outside the remit of this Panel.

2. Technical data

2.1. Identity of the substance

Ferric sodium EDTA is the monosodium salt of a chemical complex formed between ferric iron [Fe(III)] and EDTA (ethylenediaminetetraacetic acid).

The CAS Registry Number for the trihydrate form of ferric sodium EDTA is 18154-32-0, while that for the anhydrous form is 15708-41-5.

The molecular formula of the trihydrate form is: $C_{10}H_{12}N_2O_8FeNa \cdot 3H_2O$ and it has a molecular weight of 421.1 g/mol; the molecular weight of the anhydrous form is 376.0 g/mol.

The structural formula is shown in Figure 1:

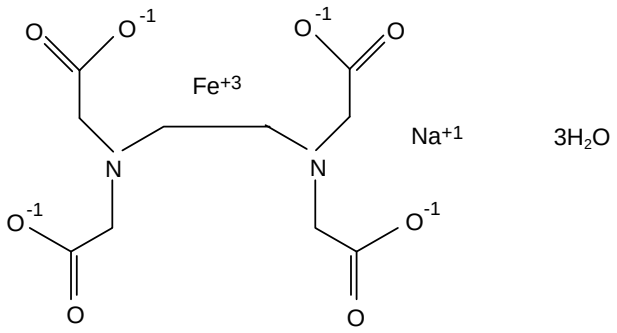

Figure 1: The structural formula of ferric sodium EDTA

The systematic names provided by the petitioner are:

Ferrate(1-), [(*N*,*N'*-1,2-ethanediylbis(N-[(carboxy-kappaO)methyl)glycinato-kappaN,kappaO)]4-)]-, sodium, (OC-6-21)

Ferrate(1-), [[*N*,*N'*-1,2-ethanediylbis[N-(carboxymethyl)glycinato]](4-)-*N*,*N'*,*O*,*O'*,*ON*,*ON'*]-, sodium, (OC-6-21)

Synonyms: The literature lists 35 synonyms for ferric sodium EDTA (ChemIDplus Advanced). Some synonyms are given below:

Ferrate(1-), [(ethylenedinitrilo)tetraacetato]-, sodium; Ferric ethylenediaminetetraacetic acid, sodium salt; Ferric sodium edentate; Iron monosodium EDTA; Iron sodium ethylenediaminetetraacetate

(1:1:1); Sodium [(ethylenedinitrilo)tetraacetato]ferrate(III); Sodium (*N*,*N*,*N'*,*N'*-ethylenediamine-tetraacetato)ferrate(1-); Sodium ferric ethylenediaminetetraacetate; Sodium iron(III) ethylenediamine-tetraacetate; Sodium [(ethylenedinitrilo)tetraacetatato]ferrate(III).

The effectiveness of EDTA as a chelator for a particular metal ion depends on its stability constant with the metal ion. This is affected by pH, the molar ratio of the chelator-to-metal ion and the presence of competing metal ions capable of forming complexes with EDTA. Of the nutritionally important metals, ferric ion (Fe^{+3}) has the highest stability constant (log k = 25.1). The optimal pH for chelate formation for ferric ion is pH 1 (Plumb *et al.*, 1950; Martel, 1960; Hart, 1984).

2.2. Specifications

Ferric sodium EDTA is an odourless free-flowing, yellow-brown powder. The petitioner states that ferric sodium EDTA, isolated via crystallisation in the trihydrate form, has a minimum chemical purity of 99 % (w/w).

The specifications as provided by the petitioner are given in Table 1. The petitioner indicates that the proposed chemical specifications for ferric sodium EDTA are consistent with those established by JECFA (JECFA, 1999).

Table 1: Chemical Specifications for ferric Sodium EDTA as proposed by the petitioner

Specification Parameter	Specification Value(1)
Solubility	Soluble in water as follows: 90 g/L water at 20°C; 120 g/L water at 30°C; 300 g/L water at 70°C
Iron	Not less than 12.5% and not more than 13.5%
Sodium	5.5%
Water	12.8%
Organic matter (CHNO)	68.4%
EDTA	Not less than 65.5 % and not more than 70.5%
pH	3.5 to 5.5 (1 in 100 solution)
Water insoluble matter	Not more than 0.1%
Nitrilo-triacetic acid	Not more than 0.1%
Arsenic	Not more than 1 mg/kg
Lead	Not more than 1 mg/kg

(1) Values obtained following chemical product analyses for 5 non-consecutive manufacturing lots of ferric sodium EDTA.

The Panel notes that according to Commission Regulation (EC) No 629/2008, the maximum levels of lead, mercury and cadmium in food supplements, as sold, should be 3.0 mg/kg, 0.1 mg/kg and 1 mg/kg, respectively (Directive 2008/128/EC)[7].

The petitioner also provided microbiological data showing that, based on an analysis of 4 non-consecutive lots of ferric sodium EDTA using standard ISO methods, no typical foodborne microorganisms are present in the final product.

2.3. Manufacturing process

Ferric sodium EDTA is obtained by crystallisation following the addition of an aqueous solution of $FeCl_3$ to an aqueous solution of tetrasodium EDTA.

2.4. Methods of analysis in food

Iron in ferric sodium EDTA added to a foodstuff may be determined using standard flame atomic absorption spectrometry. A detailed description of the method is provided by the petitioner. The estimated limit of quantification (LOQ) for the method is 3 mg/kg, with an estimated limit of detection (LOD) of approximately 1 mg/kg.

EDTA in foods can be determined using capillary electrophoresis (CE) as described by Kvasnička and Míková (1996) and by Sheppard and Henion (1997). Alternative methods for the determination of EDTA in food have been described by Krokidis *et al.* (2005) using Ion Chromatography and by Cagnasso *et al.* (2007) using High-Performance Liquid Chromatography.

2.5. Stability, reaction and fate in foods

The petitioner provides data showing that chemically pure ferric sodium EDTA in crystalline form is stable over storage periods of at least 3 years.

The photostability/photodegradation of ferric sodium EDTA has been investigated. Fidler *et al.* (2004) studied the photostability of ferric sodium EDTA in fish sauce, soy sauce and water (iron concentration: 500 mg iron/L) under various conditions of storage [clear and amber glass bottles, as well as polyethylene terephthalate (PET) bottles]. Ferric sodium EDTA in aqueous solution was found to be stable following storage in amber glass bottles for 51 days under artificial sunlight. Up to 35 % of ferric sodium EDTA in fortified fish sauce was degraded within 2 to 6 weeks of storage in clear glass bottles and exposure to direct sunlight. However, these losses were prevented by storage in amber glass bottles, or by storage in clear glass bottles under indirect sunlight or in the dark. In contrast, no degradation of ferric sodium EDTA was observed following storage of fortified soy sauce in the dark or under indirect sunlight.

The photodegradation products of EDTA in aqueous solutions have been reported to be ethylenediaminetriacetate (ED3A), ethylenediaminediacetate (EDDA), and ethylenediamine-monoacetate (EDMA) (Lockhart and Blakeley, 1975). EDMA can further degrade to iminodiacetate (IMDA), glycine, carbon dioxide, and formaldehyde, although the final product of photodegradation

[7] EC, 2008. Commission Regulation (EC) No 629/2008 of 2 July 2008 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs.

is reported to be EDMA (Nowack and Baumann 1998). According to Fidler *et al.* (2004), the progressive loss of carboxylic acid groups from EDTA to give ED3A, EDDA, and EDMA would presumably reduce its mineral binding capacity. However, no precipitation in stored fish sauce was observed, which would be expected to occur if unbound iron were present. Therefore, according to these authors, it is unlikely that 35% degradation of ferric sodium EDTA would have a major impact on iron absorption because EDTA-to-iron molar ratios of 0.5:1 to 0.7:1 have been reported to be as effective as a 1:1 molar ratio in enhancing iron absorption from moderately inhibitory meals (Hurrell *et al.,*1994; Hurrell *et al.,* 2000). Therefore, the authors state that there is no evidence to suspect ED3A, EDDA, EDMA, or IMDA to have any anti-physiological action.

As indicated above, EDMA can further degrade to carbon dioxide and formaldehyde, which are normal products of photolytic reactions (Nowack and Baumann, 1998).

2.6. Case of need and proposed uses

The petitioner justifies the need for the use of ferric sodium EDTA based on data in the literature showing that the product provides a highly bioavailable and stable source of iron in foods without significant alterations in the organoleptic properties of the food to which it is added (Viteri *et al*., 1978; Martínez-Torres *et al*., 1979; Solomons *et al*., 1979; MacPhail *et al*., 1981; Davidsson *et al*., 1994; Hurrell, 1997; Bothwell and MacPhail, 2004; Mendoza *et al*., 2004).

Proposed use in foods for particular nutritional uses (PARNUTS)

Ferric sodium EDTA is intended for use as a direct replacement for currently permitted iron forms in all foods for Particular Nutritional Uses (PARNUTS) categories, with the exception of baby foods and infant formulae (Council Directive 89/398/EEC) (Council of the European Communities, 1989).

The petitioner states that under the conditions of the intended use, the daily intake of iron from ferric sodium EDTA would not exceed the levels anticipated through existing iron supplementation programmes. The levels of addition of ferric sodium EDTA would be similar to other forms of ferric iron currently approved for use in PARNUTS foods (*i.e.*, ferric ammonium citrate, ferric pyrophosphate, ferric saccharate and ferric sodium diphosphate).

Proposed use in Food Supplements

Ferric sodium EDTA is intended for use as a source of ferric iron in food supplements (Directive 2002/46/EC)[8].

According to the petitioner, the levels of ferric sodium EDTA added to food would be similar to those of other forms of ferric iron currently approved for use in food supplements (*i.e.*, ferric ammonium citrate, ferric pyrophosphate, ferric saccharate, and ferric sodium diphosphate).

Proposed use in fortified foods

The petitioner also proposes the use of ferric sodium EDTA as a source of iron in fortified foods. The proposed use level of ferric sodium EDTA in fortified foods is 15.8 mg/serving size, which is equivalent to 2.1 mg iron/serving size. This proposed use level is equivalent to 15% of the Reference Labelling Value (RLV) for iron in the EU, i.e. 14 mg/person/day (SCF, 2003). The intended conditions of use of ferric sodium EDTA in fortified foods, and the corresponding use levels of iron

[8] Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements.

and EDTA for all typical examples of fortified food-uses of ferric sodium EDTA in the EU are summarised in Table 2.

Table 2: Summary of the individual typical fortified food uses and use levels for ferric sodium EDTA and the corresponding use levels for iron and EDTA as proposed by the petitioner

Food Category	Typical examples of Fortified Food-Uses	Serving Size[1]	Ferric Sodium EDTA		Iron		EDTA	
			Use Level[2] (mg/serving size)[3]	Use Level (%)	Use Level[2] (mg/serving size)[3]	Use Level (%)	Use Level[2] (mg/serving size)[3]	Use Level (%)
Beverages	Ready-to-drink and powdered soft drinks, not carbonated, regular and low calorie[4]	250 g (not canned) 330 g (canned)	15.8	0.0048 to 0.0063	2.1	0.0006 to 0.0008	11.0	0.0033 to 0.0044
Cereals and Cereal Products	Breads, excluding white bread	75 g (based on two medium-sized slices)	15.8	0.0211	2.1	0.0028	11.0	0.0147
Fat Spreads	Butter	15 g	15.8	0.1056	2.1	0.0140	11.0	0.0733
	Low- and reduced-fat spreads	15 g	15.8	0.1056	2.1	0.0140	11.0	0.0733
	Margarine	15 g	15.8	0.1056	2.1	0.0140	11.0	0.0733
Fruit and Nuts	Peanut butter	25 g	15.8	0.0633	2.1	0.0084	11.0	0.0440
Milks and Milk Products	Other milk drinks (chocolate milk, milk shakes)	200 g (milk drinks) 300 g (milk shakes)	15.8	0.0053 to 0.0079	2.1	0.0007 to 0.0011	11.0	0.0037 to 0.0055
	Yogurt drinks	200 g	15.8	0.0079	2.1	0.0011	11.0	0.0055
Miscellaneous	Condiments (bouillon cubes and other powdered condiments)	7 g	15.8	0.2262	2.1	0.0300	11.0	0.1571
	Soups, instant	8 g	15.8	0.1979	2.1	0.0263	11.0	0.1375

Food Category	Typical examples of Fortified Food-Uses	Serving Size[1]	Ferric Sodium EDTA		Iron		EDTA	
			Use Level[2] (mg/serving size)[3]	Use Level (%)	Use Level[2] (mg/serving size)[3]	Use Level (%)	Use Level[2] (mg/serving size)[3]	Use Level (%)
	Savoury sauces (soy, fish, Teriyaki, Hoisin, and sweet and sour sauces)	5 g (soy, fish, Hoisin) 32 g (sweet and sour)	15.8	0.0495 to 0.3167	2.1	0.0066 to 0.0420	11.0	0.0344 to 0.2200
Sugar, Preserves, and Confectionery	Chocolate bars	50 g	15.8	0.0317	2.1	0.0042	11.0	0.0220
	Chocolate spreads	25 g	15.8	0.0633	2.1	0.0084	11.0	0.0440
	Jam and fruit spreads	15 g	15.8	0.1056	2.1	0.0140	11.0	0.0733

[1] Based on average or medium portion sizes, adapted from FSA (2002)
[2] Based on 15% of the RLV for iron in the EU
[3] When a range of values is reported for a typical example fortified food-use, particular foods within that food-use may differ with respect to their serving size.
[4] It should be noted that while no food codes were identified in the U.K. NDNS for the typical example fortified food-use of ferric sodium EDTA in powdered soft drinks, the consumption from this category would be properly accounted for in the estimation of intake of ready-to-drink (or reconstituted), non-carbonated soft drinks.

2.7. Information on existing authorisations and evaluations

The use of ferric sodium EDTA as a source of iron in foods and food supplements is currently not permitted in the EU.

According to Directive 95/2/EC[9], a comparable EDTA salt [calcium disodium EDTA (E 385)], is currently approved for use as an additive in low-fat margarine, canned and bottled crustaceans and molluscs, canned and bottled fish, frozen and deep-frozen crustaceans, emulsified sauces, and canned and bottled pulses, legumes, mushrooms and artichokes (Directive 95/2/EC).

In 1992, JECFA provisionally approved the use of ferric sodium EDTA in supervised food fortification programmes, under the condition that additional studies would be conducted to assess the site of deposition of iron administered as ferric sodium EDTA and the metabolic fate of ferric sodium EDTA (JECFA, 1993).

In 1999, JECFA re-evaluated ferric sodium EDTA and concluded that the use of ferric sodium EDTA as a nutritional supplement in foods is considered acceptable when used in supervised food fortification programmes providing iron intakes of approximately 0.2 mg/kg bw/day from fortified foods (JECFA, 2000). This restriction of use in supervised fortification programmes resulted from the mandate received from the Codex Committee on Food Additives and Contaminants (CCFAC), who requested an evaluation for this specific purpose.

[9] European Parliament, The Council of the European Union. European Parliament and Council Directive No 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners. Off J Eur Communities L061, 1-53, 1995.

In 2007, the JECFA evaluated ferric sodium EDTA and concluded that it is suitable for use as a source of iron for food fortification to fulfil nutritional iron requirements. However, the total intake of iron from all food sources, including contaminants, should not exceed the PMTDI of 0.8 mg/kg bw/day. Furthermore, the JECFA stated that the total intake of EDTA should not exceed acceptable levels (not further specified).
From the ADI of 0 - 2.5 mg/kg bw/day for calcium disodium EDTA the JECFA calculated an equivalent intake value for EDTA of up to 1.9 mg EDTA/kg bw/day (JECFA, 2007).

In 1966 and in 1974, the JECFA evaluated calcium disodium EDTA as a food additive and derived an ADI for this substance of 0-2 mg/kg bw/day (JECFA, 1966; JECFA, 1974)

In 1977 and in 1990, the Scientific Committee for Food evaluated calcium disodium EDTA as an antioxidant and endorsed the ADI established by the JECFA (SCF, 1977; SCF, 1990).

The use of calcium disodium EDTA and disodium EDTA as direct additives to foods is permitted in North and South America, Asia (*e.g.*, Malaysia, Philippines), Africa, and Australia (INACG, 1993; CFR, 2005).

Ferric sodium EDTA is currently listed in the British Pharmacopoeia (BP) as a source of iron for the treatment of iron-deficiency anaemia (Sweetman, 2002). It is recommended for use orally at doses of up to 1.42 g iron/person/day, which would provide approximately 205 mg iron/person/day (Sweetman, 2002). The petitioner states that ferric sodium EDTA is used as an active pharmaceutical ingredient in commercial products in France, the UK, and Sweden.

In 2004, ferric sodium EDTA was accepted as Generally Recognized as Safe (GRAS) for use as a source of dietary iron for food fortification purposes in powdered meal replacements, flavoured milk, and fruit-flavoured beverages at a use level not exceeding 2.5 mg of iron per 200 mL of reconstituted beverage (GRAS Notice No. GRN 000152) (FDA, 2004).

In 2006, ferric sodium EDTA was accepted as GRAS for use in the iron fortification of soy, fish, teriyaki, and hoisin sauces at a level of 0.024 % iron by weight and in sweet and sour sauce at a level of 0.012 % iron by weight (FDA, 2006).

In 1993, the Scientific Committee on Food (SCF) recommended daily intakes of iron of 6 mg and 4 mg for infants aged 0.5-1 year and 1-3 years respectively, assuming 15% absorption of the daily intake. For adults, assuming 10% absorption, the recommended dietary iron intake has been estimated as between 8 and 10 mg iron/day (SCF, 1993).

In 2003, the Expert Group on Vitamins and Minerals (EVM) concluded that there were insufficient appropriate data to establish a Safe Upper Level for iron. For guidance purposes only, a supplemental intake of approximately 17 mg iron/day (equivalent to 0.28 mg/kg bw/day for a 60 kg adult) would not be expected to produce adverse effects in the majority of people (EVM, 2003).

In 2005, the EFSA Scientific Panel on Dietetic Products, Nutrition and Allergies (NDA Panel) evaluated the tolerable upper intake level (UL) for sodium. The Panel concluded that the available data were not sufficient to establish an upper level (UL) for sodium from dietary sources. The Panel stressed that there is strong evidence that the current mean levels of sodium consumption in European countries, evaluated at about 3-5 g (about 8-11g salt), are well in excess of dietary needs (about 1.5 g sodium/day in adults) and do contribute to increased blood pressure in the population, which in turn has been directly related to the development of cardiovascular disease and renal disease (EFSA, 2005).

2.8. Exposure

The petitioner provided information on the estimated exposure to both iron and EDTA from foods in the EU.

As regards iron exposure, the SCF reported that average and 97.5th percentile iron intakes from food in European countries vary from 10 to 17 mg/day and 17 to 29 mg/day respectively. Including the intake from food supplements high percentile values are in the range from 27 to 72 mg/day (SCF 2003).

The petitioner indicates that ferric sodium EDTA will be used in PARNUTS to provide 22.3 mg iron/day for a 60 kg adult and 11.1 mg iron /day for a 30 kg child. To provide these levels of iron, equivalent values of ferric sodium EDTA of about 168 mg/day and 84 mg/day respectively will be needed.

In the case of food supplements, the petitioner did not indicate precise use levels, but states that these are similar to that of other forms of iron currently approved for use in food supplements. The petitioner expects the iron intake from the use of ferric sodium EDTA not to exceed 22.3 mg/day for a 60 kg adult or 11.1 mg/day for a 30 kg child. Equally to the use of ferric sodium EDTA as a source of iron in PARNUTS, to provide these levels of iron in the case of food supplements, equivalent values of ferric sodium EDTA of about 168 mg/day and 84 mg/day respectively will be needed. It is also noted by the petitioner that food supplements containing ferric sodium EDTA as a source of iron would be consumed exclusive of PARNUTS products containing the source and vice versa, but not in combination. Nonetheless, the Panel calculated the potential exposure from all three sources.

The Panel notes that the EVM has stated that for guidance purposes only, a supplemental intake of approximately 17 mg iron/day (equivalent to 0.28 mg/kg bw/day for a 60 kg adult) would not be expected to produce adverse effects in the majority of people. An amount of 17 mg iron/day would be provided by 128.3 mg ferric sodium EDTA providing 89 mg EDTA, equivalent to about 1.5 mg EDTA/kg bw/day for an adult and 5.9 mg EDTA/kg bw/day for children weighing 15kg.

Based on these intake values the Panel calculated the resulting intake of EDTA if all iron were to be provided by ferric sodium EDTA. In the case of PARNUTS the exposure to EDTA would amount to about 116 mg/day for adults and to about 58 mg/day for children. In the case of food supplements the exposure to EDTA would also amount to 116 mg/day for adults and to about 58 mg/kg bw/day for children. The Panel notes that in both cases the exposure to EDTA will be about 1.9 mg EDTA/kg bw/day for adults and 3.9 mg EDTA/kg bw/day for children weighing 15 kg.

In the case of food fortification, the petitioner reports intake data for those foods typically being fortified with ferric sodium EDTA as listed in table 2 based on food consumption data from the NDNS programme of the UK Food Standards Agency and the UK Department of Health. Based on the calculations by the petitioner, the additional intake of iron would vary according to age between 2.2 mg/day (young children) to 4.8 mg/day (male adults) on average and between 4.8 mg/day and 11.3/day, respectively, at the 95th percentile. On a body weight basis, this is equivalent to 0.15 and 0.08 mg/kg bw/day on average and 0.33 and 0.18 mg/kg bw/day. Assuming the use levels of ferric sodium EDTA as provided by the petitioner, the total exposure to EDTA would be 11.3 mg/day for children (15 kg) and 24.6 mg/day for male adults on average, and 24.6 mg/day and 58.5 mg/day, respectively, at the 95th percentile. On a body weight basis, this is equivalent to 0.8 and 0.4 mg/kg bw/day at the average, and 1.7 and 1.0 mg/kg bw/day at the 95th percentile.

The Panel notes that no ADI for EDTA has been set but that the JECFA established an ADI for calcium disodium EDTA of 2.5 mg/kg bw/day, which can be calculated to amount to 1.9 mg EDTA/kg bw/day. Calcium disodium EDTA is the only currently approved EDTA derivative in the EU.

At present only exposure to EDTA from foods is from calcium disodium EDTA (E385) in foods such as minarine (low-fat margarine), canned and bottled crustaceans and molluscs, canned and bottled fish, frozen and deep-frozen crustaceans, emulsified sauces, and canned and bottled pulses, legumes, mushrooms and artichokes. Based on the currently approved use levels of calcium disodium EDTA and the food consumption data from the NDNS the daily intake of EDTA was calculated by the petitioner to be in the range of 2.4 mg/day for young children to 4.8 mg/day for adult males on average and 8.7 mg/day to 15.8 mg/day, respectively, at the 95th percentile. This is equivalent to 0.2 mg/kg bw/day and 0.06 mg/kg bw/day on average and 0.6 mg/kg bw/day and 0.2 mg/kg bw/day at the 95th percentile.

If assumed that ferric sodium EDTA as a source for iron would be consumed from all three sources (PARNUTS, fortified foods and supplements), the combined exposure to EDTA would be 8.6 mg/kg bw/day for children and 4.2 mg/kg bw/day for adults on average and to 9.5 mg/kg bw/day for children and 4.8 mg/kg bw/day for adults at the 95th percentile. This exceeds the value for EDTA of 1.9 mg/kg bw/day derived from the ADI established for calcium disodium EDTA. The Panel cannot estimate the probability that an individual is exposed to all products where ferric sodium EDTA is intended to be added as a source for iron although it considers this as rather unlikely.

Based on a proposed use level of ferric sodium EDTA intended to provide 22.3 mg iron on a daily basis, which is equivalent to 165 mg ferric sodium EDTA, an additional exposure to 9 mg sodium would result from this compound. Compared to the typical dietary exposure to sodium in the average range of 4,500-11,000 mg/day across Europe (EFSA 2005) this additional exposure of 9 mg is considered to be irrelevant even under concurrent exposure to ferric sodium EDTA from different sources.

Table 3: Summary information on Iron intake and anticipated potential exposure to Iron and EDTA from ferric sodium EDTA

Nutrient: Iron	**Amount (mg/day)**	**Average intake (mg/day)**	**High intake (mg/day)**	**References**
Recommended daily intake	4-20			SCF 1993
Intake range from food in Europe for adults		10-17	17-29	SCF 1993
Intake range from food and supplements in Europe for adults			27-72	SCF 1993
Amount of iron added to PARNUTS for adults as indicated by petitioner	22.3			
Amount of iron added to PARNUTS for children as indicated by petitioner	11.1			
Amount of iron added to supplements for adults as indicated by petitioner	22.3			
Amount of iron added to supplements for children as indicated by petitioner	11.1			
Amount of iron added to fortified foods as indicated by petitioner		2.2-4.8	4.8-11.3	
	Amount (mg/kg bw/day)	**Average intake (mg/kg bw/day)**	**High intake (mg/kg bw/day)**	
Source: **Ferric Sodium EDTA**				

Anticipated exposure to EDTA from PARNUTS for adults	1.9			
Anticipated exposure to EDTA from PARNUTS for children	3.9			
Anticipated exposure to EDTA from supplements for adults	1.9			
Anticipated exposure to EDTA from supplements for children	3.9			
Anticipated exposure to EDTA from fortified foods for adults		0.4	1.0	
Anticipated exposure to EDTA from fortified foods for children		0.8	1.7	
Total exposure to EDTA from all sources for adults		4.2	4.8	
Total exposure to EDTA from all sources for children		8.6	9.5	

3. Biological and toxicological data

3.1. Bioavailability

Ferric iron in food is poorly absorbed by humans because it precipitates from solutions with a pH above 3.5, unless suitable complexing agents are present. Conrad and Schade (1968) and MacPhail *et al.* (1981) showed evidence that ferric ion may be partially insoluble in the upper small intestine, where non-heme iron is absorbed. When chelated with EDTA, iron (primarily ferric iron) remains in the complex under acidic conditions in the stomach. The chelate still holds the iron in solution as the pH rises in the upper small intestine, but the strength of the complex is progressively reduced allowing a partial exchange with other metals and the release of iron for absorption (INACG, 1993).

Candela *et al*. (1984) and Simpson and Peters, (1990) provided evidence that iron from ferric sodium EDTA is dissociated from the EDTA moiety in the duodenum and is available for absorption via the physiologically regulated pathways responsible for iron uptake. MacPhail et al. (1981) and Simpson and Peters (1984) showed that only a small fraction (less than 1%) of the ferric sodium EDTA complex is absorbed intact and that it is completely eliminated via the urine. An additional small fraction (estimated to be less than 5%) of the EDTA moiety itself is absorbed, presumably bound to other metals (e.g. calcium, zinc, copper) in the gastrointestinal tract, and is also completely eliminated via the urine.

Animal and in vitro studies

Yeung *et al.* (2004) investigated the absorption of iron in order to compare the down-regulation of iron absorption from ferrous sulfate and ferric sodium EDTA. Two groups of 9 male Sprague-Dawley rats were fed basal diets for a period of 29 days, while two additional groups were fed diets containing 30000 mg elemental iron/kg diet to induce iron loading over the same period. On Day 30, one group of rats fed the basal diet and one group fed the high-iron diet were administered diets providing 35 mg iron/kg diet as radiolabelled ferrous sulfate or ferric sodium EDTA. The rats were fed their respective unlabelled diets (i.e., unlabelled ferrous sulfate or ferric sodium EDTA) for another 10 days. There was no significant difference between groups in blood haemoglobin concentrations. Rats fed the high-iron diet had significantly higher tissue non-heme iron concentrations compared to those fed the basal

diet. There was no significant difference between ferrous sulfate-fed and ferric sodium EDTA-fed rats with respect to tissue non-heme iron concentrations. Among rats fed the basal diets, iron retention and absorption was significantly greater in rats fed ferrous sulfate compared to those fed ferric sodium EDTA; however, there was no significant difference in absorption between groups fed the high-iron diets. In these groups, iron absorption was significantly decreased compared to rats fed the basal diets. Yeung *et al.* (2004) concluded that absorption of iron is down-regulated in iron-loaded rats, and that ferric sodium EDTA is "no more likely than ferrous sulfate to exacerbate iron overload in subjects with adequate body iron stores."

In an *in vitro* digestion/Caco-2 cell culture model, the availabilities and dialysabilities of various iron fortificants (ferric sodium EDTA, ferrous bisglycinate, ferrous sulfate, electrolytic iron, encapsulated ferrous fumarate, or ferrous fumarate) were compared in bread and milk (Yeung *et al.,* 2002). It was found that the amount of dialysed iron from fortified milk samples was significantly higher when ferric sodium EDTA was used as the iron fortificant, than when ferrous bisglycinate, ferrous fumarate, or encapsulated ferrous fumarate were used.

In another *in vitro* digestion/Caco-2 cell culture model, Wortley *et al*. (2002) investigated, the iron bioavailabilities of ferric sodium EDTA, ferrous bisglycinate, ferric phosphate, ferrous sulfate, ferrous carbonate, encapsulated ferrous fumarate, ferrous lactate, carbonyl iron, electrolytic iron, a polysaccharide-complexed ferrous sulfate (SQM), ferric pyrophosphate as compared to reduced iron present in a wheat-based cereal used as control. All of the iron-fortified cereals were reported to exhibit an increased iron bioavailability compared to unfortified cereals. In particular, iron bioavailabilities were higher than control in following descending order: ferric sodium EDTA (291%) > ferrous bisglycinate (125%) > ferric pyrophosphate (78%) > electrolytic iron (52%) > encapsulated ferrous fumarate (30 to 35%).

Human studies

The petitioner provided extensive information on the bioavailability of iron drawn from iron fortification studies in humans.

From a number of these studies it can be deduced that iron from ferric sodium EDTA has a high bioavailability despite the presence of inhibitory factors that form insoluble complexes with iron (Viteri *et al.,* 1978; Martínez-Torres *et al.,* 1979; MacPhail *et al.,* 1981; Hurrell *et al.,* 2000; Davidsson *et al.,* 2002, 2005, Andang'o *et al.,* 2007).

In other of these studies it is shown that when dosing iron in the form of ferric sodium EDTA, this iron is 2 to 3 times more bioavailable than iron in the form of ferrous sulfate which is being used as benchmark for comparing iron bioavailability of different iron compounds (Viteri *et al*., 1978; Martínez-Torres *et al*., 1979; MacPhail *et al*., 1981; Hurrell *et al*., 2000; Bothwell and MacPhail, 2004), and that it is efficiently incorporated into haemoglobin (MacPhail *et al*., 1981).

It can also be deduced that the absorption of iron from ferric sodium EDTA is regulated physiologically by the body's iron status, in a manner similar to that for other iron compounds. Iron absorption is higher in subjects with low serum ferritin concentrations compared to those with high serum ferritin concentrations (Candela *et al*., 1984; Hallberg *et al*., 1997; Hurrell *et al*., 2000; Mendoza *et al*., 2004).

In a study by Hurell et al. (2000) a wheat-soybean infant cereal meal was given to healthy adult males. The level of iron in this meal was increased from 5 mg iron to 15 mg iron/meal, either as ferric sodium EDTA or as ferrous sulfate. This increase in the iron level did result in a percentual decrease in the level of absorbed iron both from ferric sodium EDTA and from ferrous sulfate. Thus it seems that the body maintains iron levels through certain down-regulating systems, which control the

amount of iron absorbed and protect against the possibility of iron overload as already indicated by Hallberg *et al*. (1997) and by Yeung *et al*. (2004).

Interactions with other components in the diet

A number of studies have been conducted to investigate the effect of ferric sodium EDTA on the absorption and metabolism of other nutrients in food, including zinc, copper, calcium, manganese, and magnesium. In rats, the addition of ferric sodium EDTA to the diet reportedly enhanced the absorption and retention of zinc from the diet but had no influence on the absorption, urinary excretion and retention of copper or calcium (Hurrell *et al*., 1994).

In humans, fortification of foods with ferric sodium EDTA resulted in no effect on the metabolism of zinc, calcium, and manganese (Davidsson *et al*., 1994, 1998), but increased the absorption of iron and zinc from foods with low bioavailability in mineral nutrients (Solomons *et al*., 1979; Davidsson *et al*., 1994; Mendoza *et al*., 2004). The absorption of zinc, copper, calcium, or magnesium from meals fortified with ferric sodium EDTA or ferrous sulfate plus ascorbic acid was reported to be similar (Davidsson *et al*., 2005).

3.2. Toxicological data

3.2.1. Acute oral toxicity

Ferric sodium EDTA has a low acute oral toxicity with oral LD_{50} values of 2710 to 10000 mg/kg bw (equivalent to approximately 359 to 1326 mg iron/kg bw, respectively) in male and female Sprague-Dawley rats, and 794 mg/kg bw (approximately 105 mg iron/kg bw) in male and female Kunming mice (Sichuan Provincial Sanitary and Anti-epidemic Station, 1993; Whittaker *et al.*, 2002).

3.2.2. Short-term and subchronic toxicity

In a study by the Sichuan Provincial Sanitary and Anti-epidemic Station, (1993), groups of Sprague-Dawley rats (10 to 15/sex/group) were administered 0, 10, 40, 160, or 640 mg/kg bw/day of ferric sodium EDTA in the diet for 90 days. Treatment with ferric sodium EDTA did not affect the body weights and food utilisation rates of the rats, nor did it produce a dose-related effect on haematology parameters. Female rats showed significantly decreased serum creatinine values compared to controls. Serum glucose levels of male rats administered 160 or 640 mg/kg bw/day were significantly lower compared to those of controls. Female rats fed 640 mg/kg bw/day had significantly increased relative liver weights, although their absolute liver and body weights were comparable to those of controls. Absolute and relative spleen weights of male rats fed 640 mg/kg bw/day were significantly higher compared to controls. There were no histopathological alterations noted in the heart, liver, spleen, lungs, kidneys, thyroid gland, thymus, adrenal gland, pancreas, stomach, intestines, and testis or ovaries of ferric sodium EDTA-treated rats. The authors derived a No-Observed-Adverse-Effect Level (NOAEL) of 160 mg/kg bw/day for ferric sodium EDTA in rats.

In addition the petitioner provided a short description of a second 90 day study in the rat by Su *et al*. (1999). In this study the animals received in the diet doses between 0 and up to 2500 mg ferric sodium EDTA/kg bw/day. The authors derived a No-Observed-Effect Level (NOEL) of 250 mg ferric sodium EDTA/kg bw/day based on increased organ weights and observed histopathological alterations in male and female rats as compared to the controls.

The petitioner indicates that the above-mentioned studies by Su *et al*. (1999) and the Sichuan Provincial Sanitary and Anti-epidemic Station (1993) are the only 90-day subchronic studies available

on ferric sodium EDTA. The Panel notes that only translations of unpublished reports written in Chinese are available but considers that sufficient information is provided to assess these studies. From these studies the Panel derives an overall NOAEL of 250 mg/kg bw/day

In a study by Appel *et al*. (2001) the disposition, accumulation, and toxicity of ferric sodium EDTA were studied in groups of male Sprague-Dawley rats (40/group) administered 35, 70, or 140 mg iron/kg diet as ferric sodium EDTA for a period of 61 days. For comparison purposes, another 3 groups of rats (40/group; controls) were provided with 35, 70, or 140 mg iron/kg diet as ferrous sulfate. Baseline levels of the analytical parameters were obtained following the euthanasia of 10 untreated rats at the beginning of the study period. After 31 days of feeding, 20 rats/group were killed for examination, while the remaining 20 rats/group were kept and killed after a total feeding period of 61 days. Evaluated parameters included body weight, food and iron intake, haematology, clinical chemistry values, and histopathological examinations of various organs (i.e., adrenals, brain, caecum, colon, heart, kidneys, liver, oesophagus, rectum, small intestine, spleen, stomach, testes, and thymus). No significant differences were reported in the distribution of iron between the test and control animals, with mean daily intakes for the low-, mid- and high-doses, respectively, of 2.81, 5.67, and 11.19 mg iron/kg bw for ferric sodium EDTA (approximately equivalent to 21.2, 42.7, and 84.3 mg ferric sodium EDTA/kg bw/day, respectively) and 2.84, 5.69, and 11.54 mg iron/kg bw/day for ferrous sulfate.

There were no significant differences in the mean body weights and overall mean food consumption between the test and control groups. Also, there were no compound-related histopathological changes reported in the ferric sodium EDTA-treated group of rats. No significant differences in red blood cell counts, coagulation variables, or white blood cell counts were reported after 61 days of feeding. Significant changes in clinical chemistry parameters included decreased alkaline phosphatase activity in the mid-dose ferric sodium EDTA group, as well as increased total bilirubin levels in the mid-dose ferrous sulfate group at day 32, but not at day 62 of feeding.

Decreased serum total protein, albumin and calcium levels were reported in rats provided with 84.3 mg/kg bw/day of ferric sodium EDTA in the diet for 61 days; however, due to the lack of hepatic damage in these rats, these effects were deemed by the authors not to be toxicologically significant. The authors considered 84.3 mg/kg bw/day of ferric sodium EDTA, providing 11.2 mg iron/kg bw/day to be the NOAEL.

3.2.3. Genotoxicity

The petitioner provided data on *in vitro* mutagenicity assays conducted in *Salmonella typhimurium* (TA97a, TA98, TA100, TA102, TA1535, TA1537, and TA1538 strains, doses up to 10,000 µg iron/plate, and in *Escherichia coli* (IC188 and IC203 strains, doses up to 5,000 µg per plate. No mutagenic potential in the presence or absence of metabolic activation was exhibited (Martínez *et al*., 2000; Sichuan Provincial Sanitary and Anti-epidemic Station, 1993; Dunkel *et al*., (1999).

In an *in vitro* mouse lymphoma assay (L5178Y cells) at doses of 1.3, 2.6, 162.5, or 325.0 µg iron/mL and of 0.026, 0.052, 1.625, 3.250, or 6.500 µg iron/mL in the presence and absence of S9, respectively, a weak positive response in the presence of moderate cytotoxicity was observed both in the presence or absence of S9. Therefore, the result cannot be considered as being negative (Dunkel *et al*., 1999).

In an *in vivo* mouse bone marrow assay at doses of 50, 100, 200, or 400 mg/kg bw, no mutagenic potential was exhibited (Sichuan Provincial Sanitary and Anti-epidemic Station, 1993). The tests were carried out following Organisation for Economic Co-operation and Development (OECD) Test Guidelines.

As regards EDTA itself, the Panel is aware of the EU Risk Assessment Report on EDTA issued in 2004. In this report it is noted that only a few data on the genotoxic potential of EDTA *in vitro* and *in vivo* are available. Therefore, data from structurally related EDTA sodium salts (trisodium EDTA and disodium EDTA) have been considered. "Bacterial mutation tests are negative, but mutations and DNA damage were found in mouse lymphoma cells after exposure to very high concentrations. For somatic cells in mice (bone marrow cells) negative results with respect to the endpoints micronuclei, aneuploidy and sister chromatid exchanges were described. In germ line cells negative results were obtained for induction of structural chromosomal aberrations in spermatogonia, for induction of aneuploidy in primary and secondary spermatocytes, and also for induction of dominant lethals. A positive result was obtained in a micronucleus test with spermatids, indicating that aneugenic effects may be induced in specific phases of spermatogenesis (late spermacytoge-nesis). The effect was bound to the use of an extremely high dose in the LD50 range. Since the induction of aneuploidy is based on a threshold mode-of action, the potential for induction of aneuploidy will not be expressed at low doses. Furthermore, the effects may be indirect, resulting from the lower bioavailability of essential elements.

Altogether, EDTA and its sodium salts have a low mutagenic potential at extremely high doses. On the basis of the various negative findings and the assumption of a threshold mode-of action for aneugens, it can be concluded that EDTA and its sodium salts are not mutagenic for humans." (EU RAR, 2004).

3.2.4. Chronic toxicity and carcinogenicity

No chronic toxicity or carcinogenicity studies have been conducted with ferric sodium EDTA; however, several studies have been conducted with other EDTA salts. Ferric sodium EDTA, like other EDTA-metal complexes, dissociates in the gut to a bioavailable form of iron and an EDTA salt; hence, toxicology studies of other EDTA salts are relevant when considering the safety of ferric sodium EDTA.

In a 103-week study, groups of F344 rats (50/sex/group) and groups of $B6C3F_1$ mice (50/sex/group) were administered in the diet 3750 or 7500 mg/kg of trisodium EDTA·$3H_2O$ (equivalent to 187.5 and 375 mg/kg bw/day, respectively for the F344 rats and to 562.5 and 1125 mg/kg bw/day respectively for the $B6C3F_1$ mice). In each study a control group of 20 animals/sex received a basal diet for 104 weeks. The authors concluded that trisodium EDTA, administered in the diet at levels of 3750 and 7500 mg/kg diet, was not demonstrated to be carcinogenic neither in the F344 rats nor in the $B6C3F_1$ mice. A NOAEL of 7,500 mg trisodium EDTA·$3H_2O$/kg diet (equivalent to 375 mg/kg bw/day), the highest dose tested, in case of the F344 rats and of 7500 mg trisodium EDTA.$3H_2O$/kg diet (equivalent to 1125 mg/kg bw/day), the highest dose tested, in case of the $B6C3F_1$ mice was derived. (NCI, 1977)

Oser *et al*. (1963) studied the effects of administration of 0, 50, 125 or 250 mg calcium disodium EDTA/kg bw/day in the diet of 200 Wistar rats (25/sex/group) for a period of 24 months. The rats were observed daily for clinical condition and behaviour; their body weights and food intakes were measured weekly. There were no significant differences in physical appearance or behaviour reported among any of the groups of rats. No adverse effects on growth or on body weight were noted between control and treated groups except for a significantly increased body weight gain in the mid- and high-dose in the female rats. Blood parameters were not significantly different among the various groups. Based on the results of this study the authors derived a NOAEL of 250 mg calcium disodium EDTA/kg bw/day, the highest dose tested. This study was used by the JECFA to assign an ADI for EDTA of 2.5 mg/kg bw/day calculated as calcium disodium EDTA (JECFA, 1966, 1974).

In a study by Yang (1964) 33 Wistar rats (5 groups, number and sex per group not reported) were dosed with 0, 0.5, 1.0, or 5.0% disodium EDTA in the diet for a period of 2 years (equivalent to 0,

250, 500, and 2,500 mg/kg bw/day, respectively). No significant differences were noted between treated and control on parameters of growth, food intake, haematology and ash content of the tibia and femur. No gross lesions were observed upon histopathological examination of the internal organs and tissues. Based on the results of this study, the authors derived a NOAEL of 5% disodium EDTA (equivalent to 2500 mg/kg bw/day), the highest dose tested.

As regards EDTA, in the EU Risk Assessment Report on EDTA (EU RAR, 2004) it is noted that no epidemiological studies are available for evaluating the carcinogenic potential of EDTA. A bioassay of trisodium EDTA for possible carcinogenicity was conducted by administrating of test material in the diet to Fischer 344 rats and B6C3F1 mice. The studies did not report specific data on kidney toxicity in either species. Although a variety of tumours occurred among test and control animals of both species, no tumours were related to treatment. Taking together the negative results of the carcinogenicity study and of the cell transformation assays as well as the low mutagenic potential only expressed at extremely high dose levels it can be concluded that there is no concern on a carcinogenic potential of EDTA.

3.2.5. Reproductive and developmental toxicity

According to the petitioner, another study by Sichuan Provincial Sanitary and Anti-epidemic Station (1993) is the only developmental toxicity study available on ferric sodium EDTA. As indicated above, the Panel considered that in this study sufficient information is provided for assessing the safety of ferric sodium EDTA. This study is summarised below.

Female Sprague-Dawley rats (10 or 11/group) were administered 0, 50, 200, or 800 mg/kg bw of ferric sodium EDTA by gavage on Gestation Days (GDs) 7 to 16, and necropsied on GD 20. Relative to controls, the high-dose (800 mg/kg bw/day) dams had significantly decreased body weights on GDs 16 and 20. Mid- and high-dose dams gave rise to fetuses with slightly reduced body weights and heights relative to those of controls. Fontanel sizes tended to be higher in fetuses of the mid- and high-dose groups compared to those in the control or low-dose groups; however, a clear dose-response relationship was not observed. There was no significant difference in foetal resorption between ferric sodium EDTA-treated and control groups, and the incidence of foetal mortality was not dose-related. No external malformations were observed. While the overall incidence of delayed ossification and/or sternum fusion was significantly higher in high-dose fetuses (67.3 %), lower incidences were observed in low- (10.3 %) and mid-dose (28.3 %) fetuses compared to those of controls (32.3 %). According to the authors, a NOEL of 200 mg/kg bw/day for developmental toxicity can be derived.

The petitioner also provided data on reproductive and developmental studies conducted using similar EDTA salts, such as disodium EDTA, trisodium EDTA, tetrasodium EDTA, and calcium disodium EDTA.

In a study by Schardein *et al.* (1981) on the teratogenesis of EDTA and its salts in rats, no compound-related mortality or teratogenic effects were reported when rats were dosed orally with 967 mg EDTA/kg bw/day, 1243 mg disodium EDTA/kg bw/day, 1245 mg trisodium EDTA/kg bw/day, 1340 mg calcium disodium EDTA/kg bw/day and 1374 mg tetrasodium EDTA/kg bw/day from gestation days 7 to 14. The authors considered these respective doses to be the NOAELs for these compounds.

In studies by Oser *et al.* (1963) no significant reproductive or developmental effects were observed in multiple generations of rats exposed to diets providing levels of up to 250 mg/kg bw/day of calcium disodium EDTA, which was considered to be the NOEL in this study.

3.3. Human data

The petitioner indicates that ferric sodium EDTA has been used in numerous field trials in developing countries for the iron fortification of foods. From these studies it appears that no adverse effects were reported in humans subjected to long-term ferric sodium EDTA fortification trials in which fish and soy sauces, sugar, and curry powder were fortified with ferric sodium EDTA providing levels of 4 to 15 mg iron/person/day (equivalent to 0.067 to 0.25 mg iron/kg bw/day) (Garby and Areekul, 1974; Ballot *et al.*, 1989; Viteri *et al.*, 1995; Huo *et al.*, 2002; Thuy *et al.*, 2003).

Compared to the controls, improvements in iron status indicators, including serum levels of haemoglobin and ferritin, free erythrocyte protoporphyrin (FEP), Total Iron-Binding Capacity (TIBC), and iron stores, were reported in the fortified groups (Garby and Areekul, 1974; Ballot *et al.*, 1989; Viteri *et al.*, 1995; Huo *et al.*, 2002; Thuy *et al.*, 2003).

The fastest and greatest responses to iron fortification were observed in individuals of the fortified group who were iron-deficient at the start of the fortification study, compared to their iron-repleted counterparts (Ballot *et al.*, 1989; Viteri *et al.*, 1995). Positive responses to iron fortification were reported to decrease with time, as iron deficiencies began to disappear and iron stores started to build up in the subjects (Viteri *et al.*, 1995).

The Panel notes that the Scientific Panel on Dietetic Products, Nutrition and Allergies (NDA Panel) evaluated the safety of iron in general, when present in fortified foods and food supplements. The NDA Panel concluded that the risk of adverse effects from high iron intake from food sources, including fortified foods in some countries, but excluding supplements, is considered to be low for the population as a whole, except for those homozygous for hereditary haemochromatosis (NDA, 2004)

4. Discussion

The present opinion deals with the safety of ferric sodium EDTA as a source of iron in food for the general population (including food supplements) and in foods for particular nutritional uses (PARNUTS) and with the bioavailability of iron from this source. The safety of iron itself, in term of amounts that may be consumed, is outside the remit of this Panel.

The Panel notes that information on the bioavailability of iron from ferric sodium EDTA based on iron fortification studies in humans is available. From these studies it can be deduced that iron is liberated from the complex and that it is bioavailable despite the presence of inhibitory factors in the diet that may form insoluble complexes with iron. It is further shown that iron in the form of ferric sodium EDTA is 2 to 3 times more bioavailable than iron in the form of ferrous sulfate and that it is efficiently incorporated into haemoglobin.

The Panel further notes that the absorption of iron from ferric sodium EDTA is regulated physiologically by the body's iron status, in a manner similar to that for other iron compounds and that dietary iron fortification with ferric sodium EDTA is not expected to result in iron overload in iron-repleted individuals.

The Panel also notes that studies have been conducted to investigate the effect of ferric sodium EDTA on the absorption and metabolism of other nutrients in food (i.e. zinc, copper, calcium, manganese, and magnesium) in animals (rat) and in humans (fortification studies) and that no influence has been observed.

The Panel notes that ferric sodium EDTA will be used in PARNUTS to provide 22.3 mg iron/day for a 60 kg adult and 11.1 mg iron/day for a 30 kg child. To provide these levels of iron, equivalent values of ferric sodium EDTA of respectively 168.1 mg/day and 83.7 mg/day will be needed.

In the case of food supplements, the petitioner did not indicate precise use levels, but states that these are similar to that of other forms of iron currently approved for use in food supplements. The petitioner expects the iron intake from the use of ferric sodium EDTA in supplements not to exceed 22.3 mg/day for a 60 kg adult or 11.1 mg/day for a 30 kg child.

The Panel notes that the EVM has stated that for guidance purposes only, a supplemental intake of approximately 17 mg iron/day (equivalent to 0.28 mg/kg bw/day for a 60 kg adult) would not be expected to produce adverse effects in the majority of people. An amount of 17 mg iron/day would be provided by 128.3 mg ferric sodium EDTA providing 89 mg EDTA, equivalent to about 1.5 EDTA/kg bw/day for an adult and 5.9 mg EDTA/kg bw/day for children weighing 15kg.

When the foods as listed in Table 2 are fortified with ferric sodium EDTA the additional intake calculated by the petitioner for iron would vary according to age between 2.2 mg/day (15 kg child) to 4.8 mg/day (60 kg male adults) on average and between 4.8 mg/day and 11.3 mg/day, respectively, at the 95th percentile. On a body weight basis, this is equivalent to 0.15 and 0.08 mg/kg bw/day on average and 0.33 and 0.18 mg/kg bw/day at the 95th percentile. The Panel notes that the scenario calculated for the consumption of fortified foods by the petitioner corresponds at mean or 95th percentile intake, respectively, of 1 or 2 servings/day for children and 2 or 4 servings/day for adults. The Panel considers this scenario as being realistic.

Based on these intake values the Panel calculated the resulting intake of EDTA. In the case of PARNUTS the exposure to EDTA amounts to about 116 mg/day for adults and to about 58 mg/day for children. In the case of food supplements the exposure to EDTA will also amount to 116 mg/day for adults and to about 58 mg/day for children. In both cases the exposure to EDTA will equivalent to about 1.9 mg EDTA/kg bw/day for adults and 3.9 mg EDTA/kg bw/day for children weighing 15 kg.

In the case of food fortification, assuming the use levels of ferric sodium EDTA as provided by the petitioner, the total exposure to EDTA would be 11.3 mg/day for children (15 kg) and 24.6 mg/day for male adults on average, and 24.6 mg/day and 58.5 mg/day, respectively, at the 95th percentile. On a body weight basis, this is equivalent to 0.8 and 0.4 mg/kg bw/day at the average, and 1.7 and 1.0 mg/kg bw/day at the 95th percentile.

The Panel notes that no ADI for EDTA has been set but that the JECFA established an ADI for calcium disodium EDTA of 2.5 mg/kg bw/day, which can be calculated to amount to 1.9 mg EDTA/kg bw/day. Calcium disodium EDTA is the only currently approved EDTA derivative in the EU. The Panel further notes that the JECFA stated that the total intake of EDTA should not exceed acceptable levels.

If assumed that ferric sodium EDTA as a source for iron would be consumed from all three sources (PARNUTS, fortified foods and supplements), the combined exposure to EDTA would be 8.6 mg/kg bw/day for children and 4.2 mg/kg bw/day for adults on average and to 9.5 mg/kg bw/day for children and 4.8 mg/kg bw/day for adults at the 95th percentile. This exceeds the value for EDTA of 1.9 mg/kg bw/day derived from the ADI established for calcium disodium EDTA. The Panel cannot estimate the probability that an individual is exposed to all products where ferric sodium EDTA is intended to be added as a source for iron although it considers this as rather unlikely.

The Panel calculated the current exposure to EDTA from the approved use of calcium disodium EDTA in foods. This exposure is, on average, in the range of 2.2 mg/day (0.2 mg EDTA/kg bw/day) for children and to 4.8 mg/day(0.06 mg EDTA/kg bw/day) for adult males and to 8.7 mg/day (0.6 mg EDTA/kg bw/day) and 15.8 mg/day (0.2 mg EDTA/kg bw/day) respectively, at the 95th percentile. This is below the calculated value for EDTA of 1.9 mg/kg bw/day.

The Panel notes that, according to the EU Risk Assessment Report on EDTA, the oral exposure for tooth brackets wearing children is 0.1 mg/kg bw/day (EU RAR, 2004).

The exposure to sodium resulting from ferric sodium EDTA added to foods (PARNUTS, food supplements and fortified foods) as a source for iron is considered as negligible compared to the average range of dietary exposure to sodium (9 mg/day versus 4500-11000 mg/day).

The Panel notes that two 90-day studies with ferric sodium EDTA it the rat have been provided. From the data provided the Panel derives an overall NOAEL of 250 mg ferric sodium EDTA/kg bw/day.

From a 61-day feeding study in the rat a NOAEL of 84.3 mg/kg bw/day of ferric sodium EDTA (providing 11.2 mg iron/kg bw/day) could be derived. The Panel notes that based on the results of this study, the JECFA concluded in 2000, that administration of ferric sodium EDTA in the diet would not result in a greater uptake of iron once nutritional requirements for iron were met.

The Panel notes that *in vitro* mutagenicity assays conducted in S. *typhimurium* (7 strains) and E. *coli* (2 strains) were negative, while in an *in vitro* mouse lymphoma assay a weak positive response in the presence of moderate cytotoxicity was observed. However, similar results were observed with other iron compounds in this *in vitro* mouse lymphoma assay and the effects observed with sodium iron (III) EDTA could probably be attributed to iron rather than to EDTA. Additionally, an *in vivo* mouse micronucleus assay was negative. Furthermore, in an EU Risk Assessment Report on EDTA, it is concluded that "EDTA and its sodium salts have a low mutagenic potential at extremely high doses. On the basis of the various negative findings and the assumption of a threshold mode-of action for aneugens, it can be concluded that EDTA and its sodium salts are not mutagenic for humans." Thus, the Panel considers that from the information available there is no safety concern with respect to genotoxicity of ferric sodium EDTA as a source of iron added for nutritional purposes to foodstuffs.

No chronic toxicity or carcinogenicity studies have been conducted with ferric sodium EDTA; however, several studies have been conducted with other EDTA salts (e.g. trisodium EDTA, calcium disodium EDTA and disodium EDTA). Ferric sodium EDTA, like other EDTA-metal complexes, dissociates in the gut to a bioavailable form of iron and an EDTA salt; hence, toxicology studies of other EDTA salts are relevant when considering the safety of ferric sodium EDTA. From these studies it can be concluded that EDTA salts do not raise concern with respect to carcinogenicity.

From data on developmental studies conducted in the rat using similar EDTA salts, such as disodium EDTA, trisodium EDTA, tetrasodium EDTA, and calcium disodium EDTA, no compound-related mortality, reproductive, or teratogenic effects were reported.

From a developmental toxicity study on ferric sodium EDTA in the rat the Panel derived a NOAEL of 200 mg/kg bw/day.

Ferric sodium EDTA has been used in numerous field trials on iron fortification of foods in developing countries. From these studies it appears that no adverse effects were reported in humans subjected to long-term ferric sodium EDTA fortification trials.

The Panel notes that photodegradation of EDTA can give rise to the formation of formaldehyde. The EFSA AFC Panel examined formaldehyde when used as a preservative during the manufacture and preparation of food additives and concluded that there was no evidence indicating that formaldehyde is carcinogenic by the oral route. Therefore the Panel considers that the potential formation of formaldehyde as a degradation product from EDTA is not expected to pose a safety concern in humans under the proposed conditions of use of ferric sodium EDTA.

CONCLUSIONS

The present opinion deals with the safety of ferric sodium EDTA and with the bioavailability of the iron from this source. The safety of iron itself, in term of amounts that may be consumed, is outside the remit of this Panel.

The Panel concludes that iron is bioavailable from ferric sodium EDTA.

The Panel also concludes that the use of ferric sodium EDTA as a source of iron in food is of no safety concern as long as it does not lead to an exposure to EDTA above 1.9 mg EDTA/kg bw/day.

The Panel further concludes that ferric sodium EDTA as a source of iron at the proposed use level in fortified food for the general population would not be of safety concern.

The Panel notes that when ferric sodium EDTA is used in PARNUTS or food supplements at levels which provide 22.3 mg iron/day for an adult and 11.1 mg iron/day for a child, the corresponding exposure to EDTA would be 1.9 mg EDTA/kg bw/day for adults and 3.9 mg/kg bw/day for children.

DOCUMENTATION PROVIDED TO EFSA

1. Application for the approval of Ferrazone® ferric sodium EDTA as a source of iron for use in the manufacture of PARNUTS products and food supplements. July 2006. Submitted by Akzo Nobel Functional Chemicals.

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GLOSSARY/ABBREVIATIONS

ADI	Acceptable Daily Intake
ANS	Scientific Panel on Food Additives and Nutrient Sources added to Food
CAS	Chemical Abstracts Service
CCFAC	Codex Committee on Food Additives and Contaminants
CFR	Code of Federal Regulations
EC	European Commission
ED3A	Ethylenediaminetriacetate
EDDA	Ethylenediaminediacetate
EDMA	Ethylenediaminemonoacetate
EDTA	Ethylenediaminetetraacetic Acid
EFSA	European Food Safety Authority
EU	European Union
FAO/WHO	Food and Agriculture Organization/World Health Organization
FEP	Free Erythrocyte Protoporphyrin
FSA	UK Food Standard Agency
GD	Gestation Day
GRAS	Generally Recognized as Safe
INACG	International Nutritional Anemia Consultative Group
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LOD	Limit Of Detection
LOQ	Limit Of Quantification
NDNS	National Diet and Nutrition Survey
NCI	National Cancer Institute
NOAEL	No-Observed-Adverse-Effect Level
NOEL	No-Observed-Effect Level
OECD	Organisation for Economic Co-operation and Development
PARNUTS	Foods for Particular Nutritional Purposes
PET	Polyethylene Tetraphthalate
PMTDI	Provisional Maximum Tolerated Daily Intake
RLV	Reference Labelling Value
SCF	Scientific Committee for Food
TIBC	Total Iron-Binding Capacity