APPLICATION FOR THE APPROVAL OF FERRAZONE® FERRIC SODIUM EDTA AS A SOURCE OF IRON FOR USE IN THE MANUFACTURE OF PARNUTS PRODUCTS, FOOD SUPPLEMENTS AND FORTIFIED FOODS

July 24, 2006

Revised October 5, 2007

(to include fortified food uses and to include the latest JECFA conclusions and to include published fortification trial Andang'o *et al.*, 2007)

NON-CONFIDENTIAL

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1.0 ADMINISTRATIVE DATA

1.1 Purpose of the Request

In this application, Akzo Nobel Functional Chemicals proposes to use Ferrazone® ferric sodium ethylenediaminetetraacetate trihydrate (ferric sodium EDTA) as a direct replacement for permitted forms of iron in the European Union (E.U.) within the following regulated and not yet regulated food categories:

1.1.1 PARNUTS Products

Akzo Nobel Functional Chemicals hereby requests that Ferrazone® ferric sodium EDTA be permitted for use in all categories of PARNUTS, other than baby foods and infant formula, as regulated by *Council Directive 89/398/EEC on the approximation of the laws of the Member States relating to foodstuffs intended for particular nutritional uses* (Council of the European Communities, 1989). Specifically, Ferrazone® ferric sodium EDTA is intended for use as a source of iron within the following Community legislations: foods intended for use in energy-restricted diets for weight reduction (*Commission Directive 96/8/EC)* (Commission of the European Communities, 1996); dietary foods for special medical purposes (*Commission Directive 1999/21/EC)* (Council of the European Communities, 1999); low-sodium foods, including low-sodium or sodium-free dietary salts; gluten-free foods; foods intended to meet the expenditure of intense muscular effort, especially for sportsmen; and foods for persons suffering from carbohydrate-metabolism disorders (diabetes). In addition, Akzo Nobel Functional Chemicals requests that Ferrazone® ferric sodium EDTA be added to the *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*, which lists those substances permitted for use in PARNUTS foods (Commission of the European Communities, 2001). The levels of addition of ferric sodium EDTA would be similar to other forms of oxidized iron currently approved for use in PARNUTS foods.

1.1.2 Food Supplements

In addition to PARNUTS products, Akzo Nobel Functional Chemicals hereby also seeks to gain approval for the use of Ferrazone® ferric sodium EDTA in food supplements, as regulated by *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*,

which contains permitted lists of vitamins and minerals (European Parliament and The Council of the European Union, 2002).

1.1.3 Fortified Foods

Akzo Nobel Functional Chemicals hereby also requests that Ferrazone® ferric sodium EDTA be considered for use as a food fortificant, in accordance with Regulation (EC) No 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods (European Parliament and The Council of the European Union, 2006).

1.2 Name and Address of the Petitioner and Manufacturer

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1.4 Date of Submission of the Dossier

July 24, 2006

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(to include fortified food uses and to include the latest JECFA conclusions, plus published fortification trial Andang'o *et al.*, 2007)

2.0 TECHNICAL DATA

2.1 Identity of the Source

2.1.1 Chemical Name

Ferrate(1-), [[N,N'-1,2-ethanediylbis[N-[(carboxy-kO)methyl]glycinato-kN,kO]] (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)

2.1.2 Chemical Abstract Service (CAS) Number

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

2.1.3 Synonyms, Trade Names, Abbreviations

Synonyms for ferric sodium EDTA include:

Ferrate(1-), [(ethylenedinitrilo)tetraacetato]-, sodium
Ferric ethylenediaminetetraacetic acid, sodium salt
Ferric sodium edetate
Ferric sodium ethylenediaminetetraacetate
Iron monosodium EDTA
Iron sodium ethylenediaminetetraacetate
Iron sodium ethylenediaminetetraacetate (1:1:1)
Monosodium ferric EDTA
Sodium (ethylenediaminetetraacetato)ferrate(1-)
Sodium [(ethylenedinitrilo)tetraacetato]ferrate(III)
Sodium (*N,N,N′,N′*-ethylenediaminetetraacetato)ferrate(1-)
Sodium feredetate
Sodium ferric EDTA
Sodium ferric ethylenediaminetetraacetate
Sodium iron EDTA
Sodium iron(III) EDTA
Sodium iron(III) ethylenediaminetetraacetate
Sodium [(ethylenedinitrilo)tetraacetatato]ferrate(III)
Sodium [(ethylenedinitrilo)tetraacetato]ferrate(III)

The proposed trade name for Akzo Nobel Functional Chemicals' food-grade ferric sodium EDTA formulation is Ferrazone® ferric sodium EDTA.

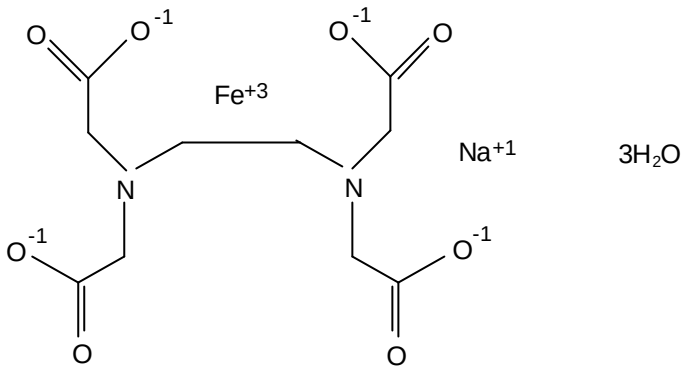
2.1.4 Constituents of the Mixture and Proportion of Each Component

Not applicable. Ferrazone® ferric sodium EDTA consists only of a single component (*i.e.*, ferric sodium EDTA trihydrate).

2.1.5 Molecular and Structural Formulae

Molecular formula: $C_{10}H_{12}N_2O_8FeNa \cdot 3H_2O$ (trihydrate)

Structural formula:



2.1.6 Molecular Weight

The molecular weight of the trihydrate form of ferric sodium EDTA is 421.1 g/mol, while that for the anhydrous form is 376.0 g/mol.

2.1.7 Spectroscopic Data

There are no available spectroscopic data for ferric sodium EDTA. See Section 2.2 for methods of identification of ferric sodium EDTA.

2.1.8 Purity

Ferric sodium EDTA is isolated *via* crystallization in the trihydrate form with a minimum chemical purity of 99% (w/w). The chemical composition (in % m/m) of ferric sodium EDTA trihydrate is as follows:

Fe	13.3
Na	5.5
H_2O	12.8
Organic matter (C, H, N, O)	68.4

2.1.9 Impurities

The primary impurities resulting from the manufacturing process include water insoluble matter (=0.1%), nitrilotriacetic acid (=0.1%), arsenic (=0.0001%), and lead (=0.0001%). Specifications for Akzo Nobel Functional Chemicals' ferric sodium EDTA include parameters for all impurities, and each batch manufactured is tested to ensure that the purity criteria are met.

2.1.10 Description of Physical State

Ferric sodium EDTA is an odourless free flowing, yellow-brown powder with the chemical composition FeNa-EDTA·$3H_2O$.

2.1.11 Solubility

Solubility in water: 90 g/L water at 20°C
120 g/L water at 30°C
300 g/L water at 70°C

2.2 Proposed Chemical Specifications and Microbiological Checkpoints

2.2.1 Chemical Specifications and Analyses for Ferric Sodium EDTA

The proposed chemical specifications for Ferrazone® ferric sodium EDTA are consistent with those established by JECFA (JECFA, 1999), and are listed in Table 2.2.1-1.

Table 2.2.1-1 Chemical Specifications for Ferrazone® Ferric Sodium EDTA

Specification Parameter	Specification Value	Analysis Method
Identification		
Solubility	Soluble in water	FNP 52/Add.7 (1999)[1]
Test for iron	Pass	FNP 52/Add.7 (1999)
Test for sodium	Pass	FNP 52/Add.7 (1999)
Purity		
Assay Iron (on a trihydrate basis)	Not less than 12.5% and not more than 13.5%	FNP 52/Add.7 (1999)
EDTA (on a trihydrate basis)	Not less than 65.5% and not more than 70.5%	FNP 52/Add.7 (1999)
PH	3.5 to 5.5 (1 in 100 solution)	FNP 52/Add.7 (1999)
Water insoluble matter	Not more than 0.1%	FNP 52/Add.7 (1999)
Nitrilotriacetic acid	Not more than 0.1%	FNP 52/Add.7 (1999)
Arsenic	Not more than 1 mg/kg	FNP 52/Add.7 (1999)
Lead	Not more than 1 mg/kg	FNP 52/Add.7 (1999)

[1] JECFA (1999)

The following chemical product analyses for 5 non-consecutive manufacturing lots (428H-021839, 428H-3091-1, 428H-3091-25, 428H-3164-4, and 428H-3165-2) of Ferrazone® ferric sodium EDTA indicate that the method of production produces a consistent product (Table 2.2.1-2). The data also support the proposed specifications, and suggest that the finished product, as produced by the manufacturing process described, is well within product specifications. See Appendix A for analytical methods.

Table 2.2.1-2 Summary of the Product Analyses for 5 Non-Consecutive Lots of Ferrazone® Ferric Sodium EDTA

Specification Parameter	Specification	Manufacturing Lot No. 428H-				
		021839	3091-1	3091-25	3164-4	3165-2
Identification						
Solubility	Soluble in water	Pass	Pass	Pass	Pass	Pass
Test for iron	Pass	Pass	Pass	Pass	Pass	Pass
Test for sodium	Pass	Pass	Pass	Pass	Pass	Pass
Purity						
Iron (%)	12.5 to 13.5	13.28	13.36	13.30	13.37	13.34
EDTA (%)	65.5 to 70.5	69.4	69.6	69.6	69.4	69.3
PH	3.5 to 5.5	4.8	4.8	4.8	4.8	4.8
Water insoluble matter (%)	Not more than 0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Nitrilotriacetic acid (%)	Not more than <0.1	<0.05	<0.05	<0.05	<0.05	<0.05
Arsenic (mg/kg)	Not more than <1	<0.1	<0.1	<0.1	<0.1	<0.1
Lead (mg/kg)	Not more than <1	<0.01	<0.01	<0.01	<0.01	<0.01

2.2.2 Additional Chemical Characterization of Ferrazone® Ferric Sodium EDTA

As a result of the presence of small amounts of residual cyanide in the starting material tetrasodium ethylenediaminetetraacetic acid, levels of cyanide are routinely monitored in different batches of Ferrazone® ferric sodium EDTA and other grades of ferric sodium EDTA (*e.g.*, Dissolvine® E-FE-13). The cyanide levels are determined using the ISO 6703 method (see Appendix A) for determination of cyanide in wastewater. Quantitation of available cyanide involves potentiometric determination with a silver ion selective electrode. This method has a level of detection of 20 mg/kg.

Residual cyanide in the EDTA reaction mixture can also react with formaldehyde to form glyconitrile that undergoes hydrolysis under alkaline conditions to form glycolate, which can be detected by high-performance liquid chromatography (HPLC). HPLC analysis of Ferrazone® ferric sodium EDTA generally does not detect quantifiable levels of glycolate, confirming that residual cyanide was not present. Additional checks for cyanide contamination are also employed during processing. Cyanide can react with iron to form Prussian Blue ($Fe_4[Fe(CN)_6]_3$), which is highly coloured and visible. A blue colour is not found in processed batches of Ferrazone® ferric sodium EDTA, which also confirms the lack of residual cyanide.

The levels of cyanide present in Ferrazone® ferric sodium EDTA and Dissolvine® E-FE-13 were determined using the ISO 6703 method, and are presented in Table 2.2.2-1. While no cyanide could be detected in manufacturing lots of Ferrazone® ferric sodium EDTA and Dissolvine® E-FE-13, approximately 70 to 80% of cyanide was recovered following the addition of 0.5 mg of cyanide to 4 g of Ferrazone® ferric sodium EDTA or Dissolvine® E-FE-13 samples (Table 2.2.2-1).

Table 2.2.2-1 Cyanide Content Data of Ferrazone® Ferric Sodium EDTA and Dissolvine® E-FE-13

Sample	Lot Number	Cyanide Addition (0.5 mg/4 g sample)	Cyanide Recovery (mg/4 g sample)	Analysis Method
Ferrazone®	428H4052-32	--	n.d.[a]	ISO 6703/2
Ferrazone®	428H4052-32	0.5	0.4	ISO 6703/2
Dissolvine® E-FE-13	428H4111-50	--	n.d.	ISO 6703/2
Dissolvine® E-FE-13	428H4111-50	0.5	0.41	ISO 6703/2
Dissolvine® E-FE-13	428H4197-50	--	n.d.	ISO 6703/2
Dissolvine® E-FE-13	428H4197-50	0.5	0.35	ISO 6703/2

[a] Not detected

Given that the ISO 6703 method has a detection limit of 20 mg/kg, and that no detectable levels of cyanide were observed in processed batches of Ferrazone® ferric sodium EDTA, then the maximum potential level of exposure to cyanide from Ferrazone® ferric sodium EDTA would be 20 mg/kg. Using the highest all-user 95th percentile intake of 100.15 mg/ person/day of Ferrazone® ferric sodium EDTA from all typical example fortified food-uses in the E.U., the maximum potential exposure to cyanide from the consumption of food products enriched with Ferrazone® ferric sodium EDTA would be approximately 0.0020 mg/person/day.

The Agency for Toxic Substances and Disease Registration (ATSDR, 2004) has determined a minimal risk level (MRL) for cyanide of 0.05 mg/kg body weight/day based on an oral no-observed-adverse-effect level (NOAEL) of 4.5 mg/kg body weight/day in an oral 90-day rat study with sodium cyanide. Thus, a 60 kg individual could consume up to 3 mg/person/day of cyanide without concern.

2.2.3 Microbiological Checkpoints and Analyses for Ferric Sodium EDTA

The proposed microbiological checkpoints for Ferrazone® ferric sodium EDTA are presented in Table 2.2.3-1.

Table 2.2.3-1 Microbiological Checkpoints for Ferrazone® Ferric Sodium EDTA		
Parameter	**Detection Limit**[1]	**Analysis Method**
Aerobic Viable Cell Count (30°C) (cfu/g)	Less than 10	Derived from Mossel and Reitsma (2001)[2]
Yeasts (cfu/g)	Less than 10	Equivalent to ISO 7954[3]
Moulds (cfu/g)	Less than 10	Equivalent to ISO 7954[3]
Pseudomonaceae (cfu/g)	Less than 50	Derived from Mossel and Reitsma (2001)
Enterobacteriaceae (37°C) (cfu/g)	Less than 10	Equivalent to ISO 7402[3]
Escherichia coli (cfu/g)	Less than 10	Equivalent to ISO 16649-2[3]
Staphylococcus aureus (cfu/g)	Less than 50	Equivalent to ISO 6888[3]
Salmonella (ELISA) (cfu/25 g)	Negative	Equivalent to NEN-EN-12824[3]

[1] For the whole sample
[2] Mossel, & Reitsma. Analytical methods for aerobic viable cell count (30°C) and *Pseudomonaceae*. In: Dijk, R.;, Grootenhuis, A., editors. *Microbiologie van Voedingsmiddelen: Methoden, Principes en Criteria*, Second Edition. Houten (The Netherlands): Keesing Noordervliet; 2001.
[3] Alcontrol Laboratories (Den Bosch, The Netherlands) method

As outlined in Table 2.2.3-2, analysis of 4 non-consecutive lots (428H-0218-14, 428H-3091-30, 428H-4260-08, 428H-5113-39) of Ferrazone® ferric sodium EDTA indicates that typical food-borne microbes are not present in the final product.

Table 2.2.3-2 Microbiological Checkpoints and Analyses for 4 Non-Consecutive Lots of Ferrazone® Ferric Sodium EDTA					
Parameter	**Detection Limit**[1]	**Lot No. 428H-**			
		0218-14	3091-30	4260-08	5113-39
Aerobic Viable Cell Count (30°C) (cfu/g)	Less than 10	<10	<10	<10	<10
Yeasts (cfu/g)	Less than 10	<10	<10	<10	<10
Moulds (cfu/g)	Less than 10	<10	<10	<10	<10
Pseudomonaceae (cfu/g)	Less than 50	<50	<50	<50	<50
Enterobacteriaceae (37°C) (cfu/g)	Less than 10	<10	<10	<10	<10
Escherichia coli (cfu/g)	Less than 10	<10	<10	<10	<10
Staphylococcus aureus (cfu/g)	Less than 50	<50	<50	<50	<50
Salmonella (cfu/25 g)	Negative	Absent	Absent	Absent	Absent

[1] For the whole sample

2.3 Manufacturing Process

2.3.1 Raw Materials Used in the Manufacturing Process

2.3.1.1 Ferric Chloride ($FeCl_3$)

Ferric chloride ($FeCl_3$) solution is prepared internally at Akzo Nobel Functional Chemicals from the naturally occurring mineral, molysite; hence, it would be considered to be technical grade material. Since $FeCl_3$ is a naturally occurring mineral, it contains a significant amount of metals, the levels of which are controlled through specification limitations. Therefore,

these metals do not carry through the production process into the final product. See Appendix B for specification details.

2.3.1.2 Tetrasodium EDTA

Tetrasodium EDTA solution is prepared internally at Akzo Nobel Functional Chemicals using the following reaction sequence:

$$EDA + 4CH_2{=}O + 4NaCN + 4H_2O \longrightarrow EDTA{-}Na_4 + 4NH_3$$

The tetrasodium EDTA solution used in the manufacturing process would be considered to be technical grade material. The production process for tetrasodium EDTA releases ammonia, which is removed from the reaction mixture. The purity of the final material is controlled by specifications that restrict the 2 major contaminants, cyanide and ammonia, to relatively low levels, which do not carry over into the final product. See Appendix B for specification details.

2.3.1.3 Hydrochloric Acid (HCl)

A 30% aqueous solution of hydrochloric acid (HCl) is used as a pH-adjusting agent in the manufacturing process of Ferrazone® ferric sodium EDTA. It is manufactured internally by Akzo Nobel Functional Chemicals, and would be equivalent to technical grade material. The levels of iron and organic compounds are controlled within the specification. See Appendix B for specification details.

2.3.2 Method of Manufacture

Ferrazone® ferric sodium EDTA is obtained by crystallization following the addition of an aqueous solution of $FeCl_3$ to an aqueous solution of tetrasodium EDTA:

$$EDTA{-}Na_4 + FeCl_3 \xrightarrow{HCl} EDTA{-}NaFe + 3NaCl$$

The production of Ferrazone® ferric sodium EDTA is conducted in accordance with the Hazard Analysis Critical Control Point (HACCP) system (see Appendix A).

2.3.3 Extraction Procedures

There are no relevant extraction procedures for ferric sodium EDTA, since it is not extracted from naturally occurring substances.

2.4 Methods of Analysis in Food

2.4.1 Analytical Methods for Determination of Iron from Ferrazone® Ferric Sodium EDTA in Foods

The levels of iron as Ferrazone® ferric sodium EDTA added to a foodstuff may be determined using the standard method of flame atomic absorption spectrometry highlighted in Akzo Nobel's Standard Method of Analysis 870.06 (see Appendix C). Representative samples of food (up to 5.0 g each) are diluted in 50.0 mL nitric acid $c(HNO_3)$ = 0.1 mol/L, homogenized, and filtered through a membrane filter. The resulting solution is then subjected to nebulization into a nitrous oxide/acetylene flame. Absorbance is measured against nitric acid using radiation emitted by an iron hollow-cathode lamp (wavelength of 248.3 nm), while correction for non-atomic absorption is performed with a deuterium lamp. The estimated limit of quantitation for this method is 3 mg/kg, implying an estimated limit of detection of approximately 1 mg/kg.

2.5 Reaction and Fate in Foods

2.5.1 Stability and Degradation or Reaction Products

Ferric sodium EDTA, being a crystalline product, is extremely stable. In July 2003, the stability of 6 samples, which had been stored as so-called "retain samples" under ambient conditions for periods of up to 4 years, was analyzed. Two samples from the year 1999 ("99A"and "99B"), 1 sample from the year 2000 ("00A"), and 3 samples from the year 2001 ("01A", "01B", and "01C") were randomly chosen. The iron content of the samples was compared to the values obtained immediately after production. The results are summarised in Table 2.5.1-1.

Table 2.5.1-1 Iron Content Data of 6 Samples of Ferrazone® Ferric Sodium EDTA

Sample Code	Iron Content (in % m/m)		Difference
	Directly after Production	Re-examined in July 2003	
99A	13.24	13.30	+ 0.06
99B	13.27	13.27	0
00A	13.34	13.30	- 0.04
01A	13.28	13.32	+ 0.04
01B	13.42	13.31	- 0.11
01C	13.36	13.31	- 0.05

Although statistical analysis of the above data has not been conducted, the results indicate that the Ferrazone® ferric sodium EDTA product is stable over storage periods of at least 3 years. Chemically pure ferric sodium EDTA, in its crystalline form, has been reported to be very stable for periods longer than the aforementioned 3 years.

Fidler *et al*. (2004) investigated the photostability of ferric sodium EDTA in fish sauce and soy sauce under various conditions of storage. Ferric sodium EDTA was added to the sauces at a concentration of 500 mg iron/L of sauce, and an aqueous solution containing the same concentration of ferric sodium EDTA was used as a control. Clear and amber glass bottles, as well as polyethylene terephthalate (PET) bottles were filled with 100 mL of ferric sodium EDTA-fortified sauces or aqueous ferric sodium EDTA solution, and subsequently stored under indirect sunlight, fluorescent light, artificial or natural sunlight, or in the dark. The concentrations of ferric sodium EDTA were measured by HPLC at regular intervals for a period of 42 days to 1 year. After approximately 2 weeks of storage of the control solution in clear glass or PET bottles under artificial or natural sunlight, ferric sodium EDTA concentrations reportedly decreased by 50 to 60%; however, no significant degradation was noted following continued storage. Exposure of the control solution-filled amber glass bottles to natural sunlight resulted in a 20% decrease in ferric sodium EDTA concentration after 42 days. Similar results were noted following storage in PET or clear glass bottles under indirect sunlight or fluorescent light; however, ferric sodium EDTA concentration was reportedly stable following storage in amber 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 bottles and exposure to direct sunlight; however, such losses were prevented by storage in amber bottles, or by storage of clear bottles under indirect sunlight or in the dark. In contrast, no degradation of ferric sodium EDTA was observed following storage of fortified soy sauce under various conditions. Table 2.5.1-2 presents the stability of ferric sodium EDTA-fortified sauces and aqueous solution under dark storage.

Table 2.5.1-2 Percentage of Ferric Sodium EDTA Remaining* in Aqueous Solution and Fortified Soy and Fish Sauces Stored in the Dark

Days	Ferric Sodium EDTA Solution	Ferric Sodium EDTA-Fortified		
		Soy Sauce	Vietnamese Fish Sauce	Thai Fish Sauce
14	100 (0.1)	100 (0.8)	100 (1.6)	100 (1.2)
28	106 (0.0)	104 (1.2)	100 (0.4)	101 (0.3)
56	106 (0.5)	100 (1.1)	100 (0.5)	97 (0.5)
112	107 (1.4)	103 (1.4)	98 (0.8)	98 (0.3)

Fidler *et al*. (2004)
* Mean (± SD), concentration of ferric sodium EDTA at baseline was 3.09 mg/mL in aqueous ferric sodium EDTA solution, 3.10 mg/mL in soy sauce, 3.23 mg/mL in Vietnamese fish sauce, and 3.25 mg/mL in Thai fish sauce.

Based on the above data, an expiry date for Ferrazone® ferric sodium EDTA of 3 years is recommended, provided it is stored in its original packaging and kept away from exposure to direct sunlight.

The photodegradation products of EDTA in aqueous solutions have been reported to be ethylenediaminetriacetate (ED3A), ethylenediaminediacetate (EDDA), and ethylenediaminemonoacetate (EDMA) (Lockhart and Blakeley, 1975). There is no evidence to suspect that ED3A, EDDA, or EDMA have any anti-physiological action (Fidler *et al*.,

2004). While the final product of EDTA photodegradation is EDMA, EDMA can further degrade to carbon dioxide and formaldehyde, which are normal products of photolytic reactions (Nowack and Baumann, 1998). Given that formaldehyde, which occurs naturally in foods, is a normal metabolite in mammals, and that a relatively high threshold exists for carcinogenicity after oral administration (Fidler *et al*., 2004), the potential formation of formaldehyde as a degradation product of EDTA is not expected to pose a safety concern in humans under the intended conditions of use of ferric sodium EDTA in foods.

2.5.2 Possible Effect of Instability on Biological Properties Including Nutrient Value

Ferric sodium EDTA has been reported not to significantly affect the organoleptic properties of fortified food products (Hodgkinson, 1961; Hurrell, 1997). In contrast to the progressive rancidity observed in wheat flour fortified with $FeSO_4 \cdot 7H_2O$ or $FeSO_4 \cdot 7H_2O$ plus disodium EDTA, fortification of wheat flour with ferric sodium EDTA did not result in fat oxidation reactions following storage at 37°C for a period of 6 months (Hurrell, 1997). Moreover, the use of ferric sodium EDTA in solution was reported to result in a palatable syrup that is void of the astringent taste or enamel-staining properties of solutions containing free ionized iron (Hodgkinson, 1961). In addition to enhancing the absorption and retention of copper, zinc, and iron from foods with low bioavailability, fortification of foods with ferric sodium EDTA produced no adverse effects on the metabolism of zinc, calcium, magnesium, and manganese in humans and rats (Solomons *et al*., 1979; Davidsson *et al*., 1994, 1998, 2005; Hurrell *et al*., 1994; Mendoza *et al*., 2004).

Following storage of soy sauce or fish sauce fortified with ferric sodium EDTA for periods of up to 1 year, no adverse alterations in taste and colour were reported (Fidler *et al*., 2004). However, ferric sodium EDTA in fish sauces could be degraded by ultraviolet rays from sunlight, resulting in the progressive loss of carboxylic acid groups from EDTA to give ED3A, EDDA, and EDMA, which could presumably reduce the mineral binding capacity of EDTA. However, no unbound iron was present in fish sauces fortified with ferric sodium EDTA, as reflected by the absence of precipitation in these sauces following up to 1 year of storage. Since molar ratios of EDTA to iron of 0.5 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 (MacPhail *et al*., 1994; Hurrell *et al*., 2000), the 35% degradation of ferric sodium EDTA observed in fish sauces stored under direct sunlight (Fidler *et al*., 2004) should not have a significant effect on their nutritional efficacy.

2.6 Case of Need and Typical example Uses

2.6.1 Justification for Ferric Sodium EDTA

By virtue of its molecular structure, ferric sodium EDTA provides a highly bioavailable and stable source of iron in foods with low iron bioavailability, without significant alterations in organoleptic properties (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). Unlike other iron compounds, which lose solubility as the pH

increases towards neutrality, the iron from ferric sodium EDTA is highly soluble, as it remains bound to EDTA in the acid milieu of the stomach, and becomes released only in the more alkaline medium of the duodenum, where it is subsequently absorbed (INACG, 1993; Hurrell, 1997; Bothwell and MacPhail, 2004). The addition of ferric sodium EDTA to meals has been shown to result in a reciprocal exchange between food iron and iron from ferric sodium EDTA, thereby enhancing the absorption of intrinsic, non-haem iron in foods (Layrisse and Martínez-Torres, 1977; Martínez-Torres *et al*., 1979; MacPhail *et al*., 1981; Hurrell, 1997). Increased absorption of zinc from foods with low zinc bioavailability also has been reported in foods fortified with ferric sodium EDTA (Solomons *et al*., 1979; Davidsson *et al*., 1994; Mendoza *et al*., 2004).

The iron from ferric sodium EDTA is absorbed by normal physiological mechanisms, such that iron overload has not been observed in iron-repleted populations (Bothwell and MacPhail, 2004). Rather, the consumption of foods fortified with ferric sodium EDTA has been consistently shown to improve the iron status of targeted human populations (*i.e.*, individuals with low iron status) in numerous fortification trials carried out in developing countries throughout the world (Garby and Areekul, 1974; Ballot *et al*., 1989a; Viteri *et al*., 1995; Mannar and Gallego, 2002; Thuy *et al*., 2003; Bothwell and MacPhail, 2004). Therefore, ferric sodium EDTA is a preferential primary candidate for use in fortification programs involving the fortification of foods with iron.

2.6.2 Intended Uses and Mode of Incorporation

2.6.2.1 PARNUTS Products

Ferrazone® ferric sodium EDTA is intended for use as a direct replacement for permitted iron forms in all PARNUTS categories with the exception of baby foods and infant formula *(Council Directive 89/398/EEC)* (Council of the European Communities, 1989).

2.6.2.2 Food Supplements

Ferrazone® ferric sodium EDTA also is intended for use as a source of ferric iron in food supplements *(Directive 2002/46/EC)* (European Parliament and The Council of the European Union, 2002*)*.

2.6.2.3 Fortified Foods

Akzo Nobel Functional Chemical also proposes the use of Ferrazone® ferric sodium EDTA as a source of iron in fortified foods, as outlined in Table 2.6.2.3-1.

Table 2.6.2.3-1 Summary of the Individual Typical Example Fortified Food-Uses for Ferrazone® Ferric Sodium EDTA in the E.U.	
Food Category	**Typical example Fortified Food-Uses**
Beverages	Ready-to-Drink and Powdered Soft Drinks, Not Carbonated, Regular and Low Calorie
Cereals and Cereal Products	Breads, Excluding White
Fat Spreads	Butter
	Low- and Reduced-Fat Spreads
	Margarine
Fruit and Nuts	Peanut Butter
Milks and Milk Products	Other Milk Drinks (Chocolate Milk, Milk Shakes)
	Yogurt Drinks
Miscellaneous	Condiments (Bouillon Cubes and Other Powdered Condiments)
	Soups, Instant
	Savoury Sauces (Soy, Fish, Teriyaki, Hoisin, and Sweet and Sour Sauces)
Sugar, Preserves, and Confectionery	Chocolate Bars
	Chocolate Spreads
	Jam and Fruit Spreads

2.6.3 Intended Use-Levels

2.6.3.1 PARNUTS Products

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

2.6.3.2 Food Supplements

As previously mentioned, the levels of addition of Ferrazone® ferric sodium EDTA would be similar to other forms of oxidized iron currently approved for use in food supplements (*i.e.*, ferric ammonium citrate, ferric pyrophosphate, ferric saccharate, and ferric sodium diphosphate).

2.6.3.3 Fortified Foods

The proposed use-levels of iron (2.1 mg/serving size) from Ferrazone® ferric sodium EDTA in fortified foods are based on 15% of the Reference Labelling Value (RLV) of iron in the E.U. of 14 mg/person/day (*SCF/CS/NUT/GEN/18 Final 6 March 2003 Opinion of the Scientific Committee on Food on the revision of reference values for nutrition labelling (expressed on 5 March 2003)* (SCF, 2003). The intended conditions of use of Ferrazone® ferric sodium EDTA in fortified foods, and the corresponding use-levels for iron and EDTA

from all typical example fortified food-uses of Ferrazone® ferric sodium EDTA in the E.U. are summarised in Table 2.6.3.3-1.

Table 2.6.3.3-1 Summary of the Individual Typical Example Fortified Food-Uses and Use-Levels for Ferrazone® Ferric Sodium EDTA and the Corresponding Use-Levels for Iron and EDTA in the E.U.

Food Category	Typical example Fortified Food-Uses	Serving size[1]	Ferrazone® 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	75 g (based on 2 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
	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

Table 2.6.3.3-1 Summary of the Individual Typical Example Fortified Food-Uses and Use-Levels for Ferrazone® Ferric Sodium EDTA and the Corresponding Use-Levels for Iron and EDTA in the E.U.

Food Category	Typical example Fortified Food-Uses	Serving size[1]	Ferrazone® 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 (%)
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 (1988)
[2] Based on 15% of the RLV for iron in the E.U.
[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 Ferrazone® 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 Exposure

2.7.1 Known Human Exposure to Iron from Food

The levels of exposure to iron from ferric sodium EDTA would comply with currently existing E.U. regulations and scientific opinions. The current Nutrition Labelling *Directive 90/496/EEC* (Council of the European Communities, 1990) indicates a recommended daily allowance (RDA) for iron of 14 mg/person/day, which was based on the *SCF 31st Series 11 Dec 1992 Nutrient and Energy Intakes for the European Community* (SCF, 1993). However, the latest Opinion from the Scientific Committee on Food (SCF) indicates a Population Reference Intake (PRI) of 9/20 and an RLV of 14 mg (*SCF/CS/NUT/GEN/18 Final 6 March 2003 Opinion of the Scientific Committee on Food on the revision of reference values for nutrition labelling (expressed on 5 March 2003)* (SCF, 2003).

Table 1 of the recently published *Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to the Tolerable Upper Intake Level of Iron (Request N° EFSA-Q-2003-018) (adopted on 19 October 2004)* indicate mean and 97.5th percentile intakes of iron ranging from 10 to 22 and 16 to 41 mg/person/ day, respectively, for populations in 8 European countries (EFSA, 2004). In this report, the European Food Safety Agency (EFSA) was unable to set a tolerable upper intake level for iron, while noting that, "the risk of adverse effects from iron overload in the general population, including those heterozygous for hereditary haemochromatosis, is considered to be low" (EFSA, 2004).

In the U.K., the Expert Group on Vitamins and Minerals (EVM) estimated mean and 97.5th percentile intakes of iron to be 12 and 24 mg/person/day, respectively (UK EVM, 2003). Including additional sources such as water and supplements, the estimated maximum intake of iron was reported to be 44 mg/person/day (UK EVM, 2003). The UK EVM (2003) also established "Guidance Levels", noting that:

> "For guidance purposes, a supplemental intake of approximately 17 mg/person/day (equivalent to 0.28 mg/kg body weight/day for a 60 kg adult) would not be expected to produce adverse effects in the majority of people. This is derived by dividing the lower end of the range found to have an effect by an uncertainty factor of 3 to allow for extrapolation from a lowest-observed-adverse-effect level (LOAEL) to a NOAEL. This is based on data referring to ferrous iron (Fe^{2+}), which is the form of iron used in supplements currently available in the United Kingdom (U.K.). No additional uncertainty factor is needed for inter-individual variation because the assessment is based on studies on large numbers of people. A safe upper level for total iron has not been estimated, as gastrointestinal effects are associated with iron in supplements rather than in foods."
>
> "The guidance value of 17 mg/person/day calculated above, does not apply to the small proportion of the population who have increased susceptibility to iron overload, *via* a mechanism of unregulated (increased) absorption from the diet, associated with

the homozygous haemochromatosis genotype (estimated prevalence of approximately 0.4% in Caucasian populations). It is not possible to give quantitative information on the difference in susceptibility between this group and normal subjects."

In 1999, JECFA considered the use of ferric sodium EDTA as a nutritional supplement in foods acceptable when used in supervised food fortification programs that would provide iron intakes of approximately 0.2 mg/kg body weight/day from fortified foods (JECFA, 2000). This restriction of use in supervised fortification programs resulted from the mandate received from the Codex Committee on Food Additives and Contaminants (CCFAC), who requested an evaluation for this specific purpose and use-level (note in 2007, JECFA re-evaluated ferric sodium EDTA - see Section 2.8).

According to the Institute of Medicine (IOM), this recommended use-level was directed towards adult women and did not include children that have an iron requirement significantly higher than this value (IOM, 2001). In numerous field trials in developing countries, ferric sodium EDTA (supplied as Ferrazone® ferric sodium EDTA) has been used for the iron fortification of foods providing 4 to 15 mg iron/day (0.067 to 0.25 mg iron/kg body weight/day) of supplemental dietary iron without any adverse effects. In particular, dietary iron fortification with ferric sodium EDTA is not expected to result in iron overload in otherwise normal populations.

The current fortification programs have used iron levels well below the tolerable intakes for iron published by JECFA (1983) and the IOM (2001). JECFA (1983) presented a provisional maximum tolerable intake of 0.8 mg/kg body weight/day based on the safe long-term intake of ferrous supplements of 48 mg/person/day (for a 60 kg individual). The IOM (2001) presented a Tolerable Upper Limit of 45 mg/person/day of iron from all sources based on gastrointestinal effects observed in individuals consuming 60 mg of iron from ferrous fumarate as supplemental iron to the diet. The intake of iron was corrected for 10 mg of dietary iron, bringing the total dietary exposure to 70 mg of iron. An uncertainty factor of 1.5 was applied to the LOAEL to obtain the Tolerable Upper Limit of 45 mg/person/day. These values are considerably lower than the apparent safe level of iron derived from rats fed ferric sodium EDTA. Thus, dietary levels of ferric sodium EDTA that provide less than 45 mg of iron/day would be considered safe for humans.

2.7.2 Background Intake of EDTA from Permitted Food-Uses in the E.U.

In addition to food categories in which Ferrazone® ferric sodium EDTA is typical example for use, the exposure to EDTA would also be anticipated through the consumption of commercially available foods fortified with calcium disodium EDTA. Calcium disodium EDTA (E385) is currently approved for use in the E.U. *(Directive 95/2/EC of the European Parliament and the Council of 20 February 1995 on food additives other than colours and sweeteners)* as an additive to such foods as minarine, 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 (European Parliament

and The Council of the European Union, 1995). The currently approved food-uses for calcium disodium EDTA and the corresponding use-levels for EDTA in the E.U. are summarised in Table 2.7.2-1.

Table 2.7.2-1 Summary of the Currently Approved Food-Uses of Calcium Disodium EDTA and the Corresponding Use-Levels of EDTA in the European Union

Food Category	Typical example Food-Use	EDTA Use-Level (%)
Fat Spreads	Minarine	0.007
Fish and Fish Dishes	Canned and Bottled Crustaceans and Molluscs	0.00525
	Canned and Bottled Fishes	0.00525
	Frozen and Deep-Frozen Crustaceans	0.00525
Miscellaneous	Emulsified Sauces	0.00525
Vegetables, Potatoes, and Savoury Snacks	Canned and Bottled Pulses, Legumes, Mushrooms, and Artichokes	0.0175

The consumption of EDTA from commercially available foods fortified with calcium disodium EDTA was estimated based on use-levels summarised in Table 2.7.2-1 and food consumption data collected as part of The National Diet and Nutrition Survey (NDNS) programme described in Section 2.7.3. The estimated exposure to EDTA from commercially available foods fortified with calcium disodium EDTA is summarised in Table 2.7.2-2 on an absolute basis (mg/person/day), and in Table 2.7.2-3 on a per kilogram body weight basis (mg/kg body weight/day). It should be noted that since no food codes were identified for minarine, food codes for low and very low fat spreads were used as a surrogate.

Table 2.7.2-2 Summary of the Estimated Daily Intake of EDTA in the U.K. from All Current Food-Uses of Calcium Disodium EDTA in the E.U. by Population Group (NDNS Data)[1]

Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption			All-User Consumption		
				Mean (mg)	Percentile (mg)		Mean (mg)	Percentile (mg)	
					90	95		90	95
Children	1½ to 4 ½	72.6	1,196	2.37	6.67	8.66	3.21	7.59	9.61
Young People	4 to 10	82.3	689	3.43	8.36	10.37	4.13	8.88	11.29
Female Teenagers	11 to 18	81.8	365	3.78	9.94	13.48	4.50	10.15	14.59
Male Teenagers	11 to 18	82.9	345	5.15	12.80	16.95	6.04	14.16	17.79
Female Adults	16 to 64	80.3	769	3.29	8.97	11.45	3.82	9.39	11.80
Male Adults	16 to 64	81.1	621	4.94	12.49	15.75	5.75	13.70	16.04

[1] Gregory *et al.* (1995); UKDA (1995, 2001); Office for National Statistics and FSA (2005)

Greater than 72% of the population groups consisted of users of those food products in which calcium disodium EDTA is currently permitted for use in the E.U. Of the individual population groups, on an absolute basis, male adults were determined to have the greatest mean all-user intake of EDTA from all current food-uses of calcium disodium EDTA with 5.75 mg/person/day (0.07 mg/kg body weight/day), while male teenagers had the highest

95th percentile all-user intake of EDTA with 17.79 mg/person/day (0.36 mg/kg body weight/day). Children had the lowest mean and 95th percentile all-user intakes of 3.21 and 9.61 mg EDTA/person/day, respectively, on absolute basis. On a body weight basis, children were identified as having the highest intakes of any population group, with mean and 95th percentile all-user intakes of 0.23 and 0.67 mg EDTA/kg body weight/day, respectively, while female adults had the lowest mean and 95th percentile intakes of 0.06 and 0.17 mg EDTA/kg body weight/day, respectively.

Table 2.7.2-3 Summary of the Estimated Daily Per Kilogram Body Weight Intake of EDTA in the U.K. from All Current Food-Uses of Calcium Disodium EDTA in the E.U. by Population Group (NDNS Data)[1]

Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption			All-User Consumption		
				Mean (mg/ kg bw)	Percentile (mg/kg bw)		Mean (mg/ kg bw)	Percentile (mg/kg bw)	
					90	95		90	95
Children	1½ to 4½	72.6	1,196	0.17	0.46	0.61	0.23	0.54	0.67
Young People	4 to 10	82.3	689	0.13	0.35	0.41	0.16	0.37	0.43
Female Teenagers	11 to 18	81.8	365	0.07	0.19	0.28	0.09	0.22	0.29
Male Teenagers	11 to 18	82.9	345	0.09	0.24	0.31	0.11	0.26	0.36
Female Adults	16 to 64	80.3	769	0.05	0.13	0.17	0.06	0.14	0.17
Male Adults	16 to 64	81.1	621	0.06	0.15	0.20	0.07	0.16	0.22

[1] Gregory *et al.* (1995); UKDA (1995, 2001); Office for National Statistics and FSA (2005)

2.7.3 Anticipated Human Exposure to Ferric Sodium EDTA and Iron from Typical example Uses of Ferrazone® Ferric Sodium EDTA in the E.U.

2.7.3.1 PARNUTS Products

Under the conditions of intended use of Ferrazone® ferric sodium EDTA, the daily intake of iron from Ferrazone® ferric sodium EDTA would be anticipated to be similar to that of existing iron supplementation programmes, which utilize other forms of oxidized iron currently approved for use in PARNUTS foods. PARNUTS products containing Ferrazone® ferric sodium EDTA would be consumed under the supervision of a qualified physician; thus, ensuring the proper use of the product. Therefore, the consumption of PARNUTS products containing Ferrazone® ferric sodium EDTA is expected to produce no safety concerns in humans.

Based on the acceptable daily intake (ADI) for calcium disodium EDTA of 2.5 mg/kg body weight/day, which was assigned by JECFA (1966, 1974) (see Section 3.5.4), and the molar ratio of calcium disodium EDTA to ferric sodium EDTA, the intake of iron from the use of Ferrazone® ferric sodium EDTA is expected not to exceed 22.3 mg/person/day for a 60 kg adult or teenager, or 11.1 mg/person/day for a 30 kg young person.

2.7.3.2 Food Supplements

As previously discussed, it is anticipated that the daily intake of iron from Ferrazone® ferric sodium EDTA would be similar to that of other forms of oxidized iron currently approved for use in food supplements. The intake of iron from the use of Ferrazone® ferric sodium EDTA is expected not to exceed 22.3 mg/person/day for a 60 kg adult or teenager, or 11.1 mg/ person/day for a 30 kg young person (see Section 2.7.3.1). It should be noted that food supplements containing Ferrazone® ferric sodium EDTA would be consumed exclusive of PARNUTS products containing Ferrazone® ferric sodium EDTA, and *vice versa*; not in combination. Therefore, the consumption of food supplements containing Ferrazone® ferric sodium EDTA is expected to produce no safety concerns in humans.

2.7.3.3 Fortified Foods

Estimates for the consumption of Ferrazone® ferric sodium EDTA, iron, and EDTA in the E.U. were based on typical example food-uses and proposed use-levels for Ferrazone® ferric sodium EDTA summarised in Table 2.6.3.3-1 and food consumption data collected as part of the NDNS programme, a joint initiative between the U.K. Food Standards Agency and the Department of Health. The NDNS programme was commissioned jointly in 1992 by the Ministry of Agriculture, Fisheries and Food (MAFF) and the Department of Health. MAFF's responsibility for the program was transferred to the Food Standards Agency on its inception in April 2000. The NDNS programme consists of 4 different surveys for specific age groups, conducted approximately every 3 years in succession. Separate survey data are available from the U.K. Data Archive (UKDA) for The NDNS: Adults Aged 16 to 64 years collected in 2000-2001 (NDNS 2000-2001) (Office for National Statistics and FSA, 2005), the National Diet, Nutrition and Dental Survey of Children Aged 1½ to 4½ Years, 1992-1993 (NDNS, 1992-1993) (UKDA, 1995), the National Diet and Nutrition Survey: Young People aged 4 to 18 Years (NDNS, 1997) (UKDA, 2001), and the National Diet and Nutrition Survey: People Aged 65 Years and Over, 1994-1995; however, only the former 3 surveys were used to generate estimates in the current intake analysis. Combined, these surveys provide the most up-to-date data for evaluating food-use, food-consumption patterns, and nutritional status in the U.K., containing 4- or 7-day weighed food records for individuals selected using a stratified multi-stage random probability design, with sampling of private households throughout Great Britain using postal sectors (UKDA, 1995, 2001) or local authority wards (UKDA, 1991) as the primary sampling unit.

NDNS data were collected from individuals and households *via* 4- (children, aged 1½ to 4½) or 7-day (young people, aged 4 to 18 and adults, aged 16 to 64) weighed dietary intake records throughout all 4 seasons of the year (4 fieldwork waves of 3 months duration), in order to address variability in eating behaviours due to seasonality. Dietary data were recorded by survey respondents, or in the case of the children's survey, by parents or guardians, for the duration of the survey period. The adult NDNS 2000-2001 contains 7-day weighed dietary records for 1,724 individuals aged 16 to 64 who were not pregnant or breastfeeding, while, NDNS 1992-1993 contributes 4-day data from an additional 1,592 children 1½ to 4½ years of age. NDNS 1997 adds 7-day records for approximately 1,700

youth aged 4 to 18 (UKDA, 1995, 2001; Office for National Statistics and FSA, 2005). The initial postal and interview sifts to identify eligible children, youth, or adults, respectively, for the surveys identified 93, 92, and 65% eligibility; the maximum response rate (individuals agreeing to the initial dietary interview) from the eligible sample selected for participation in the survey were, 88, 80, and 61%, respectively, while only 81, 64, and 47% of surveyed individuals, respectively, completed a full dietary record (Gregory *et al.*, 1995; UKDA, 2001; Office for National Statistics and FSA, 2005).

In addition to collecting information on the types and quantities of foods being consumed, the NDNS programme collects physiological, anthropometric and demographic information from individual survey participants, such as sex, age, measured height and weight (by the interviewer), blood analytes, and other variables useful in characterizing consumption. The inclusion of this information allows for further assessment of food intake based on consumption by specific population groups of interest within the total surveyed samples. Sample weights were developed and incorporated with the youth survey (NDNS, 1997) to compensate for the potential under-representation of intakes from specific population groups as a result of sample variability due to differential sampling probabilities and differential non-response rates, particularly the lower response obtained from males, aged 15 to 18 years (UKDA, 2001).

To facilitate comparison with the adult and youth 7-day dietary survey data, dietary data from the children's survey (4-day data) was weighted to 7 days, based on the assumption that intake patterns on non-recording weekdays were similar to dietary intakes on recorded weekdays; the 2 weekend days were not reweighted. Accordingly, all food and drink consumed on the 2 recorded weekdays were averaged to give a daily intake value, which was multiplied by 5 to approximate intakes for all weekdays. These values were then combined with consumption data from weekend dietary records. Full details of the weighting method applied are provided in Appendix J of the report on the children's diet and nutrition survey (Gregory *et al*., 1995).

Consumption data from individual dietary records, detailing food items ingested by each survey participant on each of the survey days, were collated by computer and used to generate estimates for the intake of ferric sodium EDTA and iron by the U.K. population. Estimates for the daily intake of ferric sodium EDTA and iron represent projected 7-day averages for each individual from Days 1 to 7 of NDNS data; these average amounts comprised the distribution from which mean and percentile intake estimates were produced. Mean and percentile estimates were generated using ratio estimation and nonparametric techniques, incorporating survey weights where appropriate (*i.e.,* when using youth data to estimate intakes) in order to provide representative intakes for specific U.K. population groups. All-person intake refers to the estimated intake of ferric sodium EDTA and iron averaged over all individuals surveyed, regardless of whether they consumed food products in which Ferrazone® ferric sodium EDTA is currently typical example for use, while all-user intake refers to the estimated intake of Ferrazone® ferric sodium EDTA and iron by those individuals consuming food products in which the use of Ferrazone® ferric sodium EDTA is under consideration, hence the 'all-user' designation. Individuals were considered users if

they consumed 1 or more food products in which Ferrazone® ferric sodium EDTA is typical example for use on 1 of the 7 survey days.

Calculations for the mean and high-level (95th percentile) all-person and all-user intakes, and percent consumers were performed for each of the individual typical example fortified food-uses for Ferrazone® ferric sodium EDTA. Similar calculations were used to determine the estimated total intake of iron and EDTA from all typical example fortified food-uses for Ferrazone® ferric sodium EDTA combined. In both cases, the per person and per kilogram body weight intakes were reported for the following population groups:

children, ages 1½ to 4½ years ;
young people, ages 4 to 10 years;
female teenagers, ages 11 to 18 years;
male teenagers, ages 11 to 18 years;
female adults, ages 16 to 64 years; and
male adults, ages 16 to 64 years.

2.7.3.3.1 Estimated Daily Intake of Ferrazone® Ferric Sodium EDTA from All Typical example Fortified Food-Uses in the E.U.

The estimated total consumption of Ferrazone® ferric sodium EDTA from all typical example fortified food-uses is summarised in Tables 2.7.3.3.1-1 and 2.7.3.3.1-2 on a mg/person/day and mg/kg body weight/day basis, respectively. It should be noted that while no food codes were identified in the U.K. NDNS for the typical example food-use of Ferrazone® 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. A complete description of the consumption estimates is provided in Appendix D.

As would be expected for a 7-day survey, the percentage of users was high among all age groups evaluated in the current intake assessment. Greater than 92% of the population groups consisted of users of those food products in which Ferrazone® ferric sodium EDTA is currently typical example for use. Large user percentages within a population group typically lead to similar results for the all-person and all-user consumption estimates. Consequently, only the all-user intake results will be discussed in detail.

Estimates for the intake of Ferrazone® ferric sodium EDTA in the E.U. were based on the typical example fortified food-uses and use-levels summarised in Table 2.6.3.3-1 and food consumption data collected as part of the NDNS programme. Of the individual population groups, the greatest mean and 95th percentile all-user intakes of Ferrazone® ferric sodium EDTA on an absolute basis were determined in male adults (37.18 mg/person/day) and female adults (100.15 mg/person/ day), respectively. Children had the lowest mean and 95th percentile all-user intakes of Ferrazone® ferric sodium EDTA on an absolute basis with 16.63 and 35.97 mg/person/day, respectively. Estimated daily Ferrazone® ferric sodium EDTA intakes increased with age in all groups, but were generally lower in females relative to males.

Table 2.7.3.3.1-1 Summary of the Estimated Daily Intake of Ferrazone® Ferric Sodium EDTA in the U.K. from All Typical Example Food Categories in the E.U. by Population Group (NDNS Data)

Population Group	Age Group (Years)	% User	Actual # of Total Users	All-Person Consumption			All-User Consumption		
				Mean (mg)	Percentile (mg)		Mean (mg)	Percentile (mg)	
					90	95		90	95
Children	1½ to 4½	97.6	1,609	16.46	29.16	35.97	16.63	29.16	35.97
Young People	4 to 10	99.3	831	23.31	41.54	48.81	23.41	41.97	48.81
Female Teenagers	11 to 18	97.1	433	25.98	43.61	55.07	26.27	43.61	56.27
Male Teenagers	11 to 18	98.8	411	28.92	48.92	65.88	29.01	48.57	66.21
Female Adults	16 to 64	92.5	886	33.52	57.62	99.66	34.62	59.24	100.15
Male Adults	16 to 64	93.2	714	35.98	69.02	85.52	37.18	69.66	89.36

Conversely, on a body weight basis, children were identified as having the highest intakes of any population group, with mean and 95th percentile all-user Ferrazone® ferric sodium EDTA intakes of 1.16 and 2.47 mg/kg body weight/day, respectively, while male adults had the lowest mean all-user intake of 0.45 mg/kg body weight/day, and female teenagers had the lowest 95th percentile intake of 1.02 mg/kg body weight/day.

Table 2.7.3.3.1-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Ferrazone® Ferric Sodium EDTA in the U.K. from All Typical Example Food Categories in the E.U. by Population Group (NDNS Data)

Population Group	Age Group (Years)	% User	Actual # of Total Users	All-Person Consumption			All-User Consumption		
				Mean (mg/ kg bw)	Percentile (mg/kg bw)		Mean (mg/ kg bw)	Percentile (mg/kg bw)	
					90	95		90	95
Children	1½ to 4½	97.6	1,609	1.13	1.97	2.46	1.16	2.00	2.47
Young People	4 to 10	99.3	831	0.90	1.63	1.93	0.91	1.63	1.94
Female Teenagers	11 to 18	97.1	433	0.49	0.88	1.02	0.50	0.89	1.02
Male Teenagers	11 to 18	98.8	411	0.54	0.96	1.22	0.54	0.97	1.23
Female Adults	16 to 64	92.5	886	0.47	0.94	1.34	0.51	0.97	1.49
Male Adults	16 to 64	93.2	714	0.42	0.80	1.05	0.45	0.82	1.07

2.7.3.3.2 Estimated Daily Intake of Iron from All Typical example Fortified Food-Uses of Ferrazone® Ferric Sodium EDTA in the E.U.

Estimates for the intake of iron in the E.U. were based on the typical example fortified food-uses and use-levels of Ferrazone® ferric sodium EDTA summarised in Table 2.6.3.3-1 and food consumption data collected as part of the NDNS programme. Consistent with the discussion on the intakes of Ferrazone® ferric sodium EDTA, only the all-user intake results will be discussed in detail. On an absolute basis, male adults were determined to have the

greatest mean all-user intake of iron from all typical example fortified food-uses of Ferrazone® ferric sodium EDTA with 4.93 mg/person/day, while female adults had the highest 95th percentile all-user intake of iron with 13.28 mg/person/day (Table 2.7.3.3.2-1). Children had the lowest mean and 95th percentile all-user intakes, at 2.21 and 4.77 mg/person/day, respectively. Estimated daily iron intakes increased with age in all groups, but were generally lower in females relative to males.

Table 2.7.3.3.2-1 Summary of the Estimated Daily Intake of Iron in the U.K. from All Typical Example Fortified Food-Uses of Ferrazone® Ferric Sodium EDTA in the E.U. by Population Group (NDNS Data)

Population Group	Age Group (Years)	% User	Actual # of Total Users	All-Person Consumption			All-User Consumption		
				Mean (mg)	Percentile (mg)		Mean (mg)	Percentile (mg)	
					90	95		90	95
Children	1½ to 4½	97.6	1,609	2.18	3.87	4.77	2.21	3.87	4.77
Young People	4 to 10	99.3	831	3.09	5.51	6.47	3.11	5.57	6.47
Female Teenagers	11 to 18	97.1	433	3.45	5.78	7.30	3.48	5.78	7.46
Male Teenagers	11 to 18	98.8	411	3.84	6.49	8.74	3.85	6.44	8.78
Female Adults	16 to 64	92.5	886	4.45	7.64	13.22	4.59	7.86	13.28
Male Adults	16 to 64	93.2	714	4.77	9.15	11.34	4.93	9.24	11.85

On a body weight basis, children were identified as having the highest intakes of any population group, with mean and 95th percentile all-user iron intakes of 0.15 and 0.33 mg/kg body weight/day, respectively, while male adults had the lowest mean all-user intake of 0.06 mg/kg body weight/day (Table 2.7.3.3.2-2). The lowest 95th percentile all-user intakes of iron from all typical example fortified food-uses of Ferrazone® ferric sodium EDTA were determined in female teenagers and male adults, at 0.14 mg/kg body weight/day.

Table 2.7.3.3.2-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Iron in the U.K. from All Typical Example Fortified Food-Uses of Ferrazone® Ferric Sodium EDTA in the E.U. by Population Group (NDNS Data)

Population Group	Age Group (Years)	% User	Actual # of Total Users	All-Person Consumption			All-User Consumption		
				Mean (mg/ kg bw)	Percentile (mg/kg bw)		Mean (mg/ kg bw)	Percentile (mg/kg bw)	
					90	95		90	95
Children	1½ to 4½	97.6	1,609	0.15	0.26	0.33	0.15	0.26	0.33
Young People	4 to 10	99.3	831	0.12	0.22	0.26	0.12	0.22	0.26
Female Teenagers	11 to 18	97.1	433	0.06	0.12	0.14	0.07	0.12	0.14
Male Teenagers	11 to 18	98.8	411	0.07	0.13	0.16	0.07	0.13	0.16
Female Adults	16 to 64	92.5	886	0.06	0.12	0.18	0.07	0.13	0.20
Male Adults	16 to 64	93.2	714	0.06	0.11	0.14	0.06	0.11	0.14

Based on the ADI for calcium disodium EDTA of 2.5 mg/kg body weight/day, which was assigned by JECFA (1966, 1974) (see Section 3.5.4), and the molar ratio of calcium disodium EDTA to ferric sodium EDTA, the intake of iron from the use of Ferrazone® ferric sodium EDTA is expected not to exceed 22.3 mg/person/day for a 60 kg adult or teenager, 11.1 mg/person/day for a 30 kg young person, or 1.9 mg/person/day for a 5 kg child. With the exception of children, actual intakes of iron from all typical example fortified food-uses of Ferrazone® ferric sodium EDTA at the 95th percentile for the corresponding population groups in the U.K. are below these levels (Table 2.7.3.3.2-1). Although the estimated 95th percentile all-user intake of 4.77 mg iron/day in children (Table 2.7.3.3.2-1) from all typical example fortified food-uses of Ferrazone® ferric sodium EDTA is higher than the calculated maximum intake of iron from Ferrazone® ferric sodium EDTA (1.9 mg/person/day for a 5 kg child), it is still below the RLV of 8 mg for children, as indicated by the latest Opinion from the SCF (*SCF/CS/NUT/GEN/18 Final 6 March 2003 Opinion of the Scientific Committee on Food on the revision of reference values for nutrition labelling (expressed on 5 March 2003)* (SCF, 2003).

2.7.3.4 Estimated Daily Intake of EDTA From All Typical Example Fortified Food-Uses of Ferrazone® Ferric Sodium EDTA in the E.U.

Estimates for the consumption of EDTA from all typical example fortified food-uses and use-levels for Ferrazone® ferric sodium EDTA in the E.U. are summarised in Tables 2.7.3.3.3-1 and 2.7.3.3.3-2 on a mg/day and mg/kg body weight/day basis, respectively. Consistent with the discussion on the intakes of Ferrazone® ferric sodium EDTA, only the all-user intake results will be discussed in detail. Of the individual population groups, the greatest mean and 95th percentile all-user intakes of EDTA on an absolute basis were determined in male adults (25.45 mg/ person/day) and female adults (68.54 mg/person/day), respectively. Children had the lowest mean and 95th percentile all-user intakes of EDTA on an absolute basis with 11.38 and 24.62 mg/person/day, respectively. Estimated daily EDTA intakes increased with age in all groups, but were generally lower in females relative to males.

Table 2.7.3.3.3-1 Summary of the Estimated Daily Intake of EDTA in the U.K. from All Typical Example Fortified Food-Uses of Ferrazone® Ferric Sodium EDTA in the E.U. by Population Group (NDNS Data)

Population Group	Age Group (Years)	% User	Actual # of Total Users	All-Person Consumption			All-User Consumption		
				Mean (mg)	Percentile (mg)		Mean (mg)	Percentile (mg)	
					90	95		90	95
Children	1½ to 4½	97.6	1,609	11.27	19.96	24.62	11.38	19.96	24.62
Young People	4 to 10	99.3	831	15.95	28.43	33.41	16.03	28.73	33.41
Female Teenagers	11 to 18	97.1	433	17.78	29.85	37.69	17.98	29.85	38.51
Male Teenagers	11 to 18	98.8	411	19.80	33.48	45.09	19.86	33.24	45.32
Female Adults	16 to 64	92.5	886	22.94	39.44	68.21	23.70	40.54	68.54
Male Adults	16 to 64	93.2	714	24.63	47.24	58.53	25.45	47.68	61.16

Children were identified as having the highest intakes of any population group, on a body weight basis, with mean and 95th percentile all-user EDTA intakes of 0.79 and 1.69 mg/kg body weight/day, respectively. Male adults had the lowest mean all-user intake (0.31 mg/kg body weight/day), while female teenagers had the lowest 95th percentile all-user intake (0.70 mg/kg body weight/day) of EDTA from all typical example fortified food-uses of Ferrazone® ferric sodium EDTA in the E.U.

Table 2.7.3.3.3-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of EDTA in the U.K. from All Typical Example Fortified Food-Uses of Ferrazone® Ferric Sodium EDTA in the E.U. by Population Group (NDNS Data)

Population Group	Age Group (Years)	% User	Actual # of Total Users	All-Person Consumption			All-User Consumption		
				Mean (mg/ kg bw)	Percentile (mg/kg bw)		Mean (mg/ kg bw)	Percentile (mg/kg bw)	
					90	95		90	95
Children	1½ to 4½	97.6	1,609	0.77	1.35	1.68	0.79	1.37	1.69
Young People	4 to 10	99.3	831	0.62	1.11	1.32	0.62	1.12	1.33
Female Teenagers	11 to 18	97.1	433	0.33	0.60	0.70	0.34	0.61	0.70
Male Teenagers	11 to 18	98.8	411	0.37	0.66	0.84	0.37	0.66	0.84
Female Adults	16 to 64	92.5	886	0.32	0.64	0.92	0.35	0.66	1.02
Male Adults	16 to 64	93.2	714	0.29	0.54	0.72	0.31	0.56	0.73

2.7.3.3.4 Estimated Daily Intake of EDTA from All Typical Example Fortified Food-Uses of Ferrazone® Ferric Sodium EDTA and All Permitted Food-Uses of Calcium Disodium EDTA in the E.U.

The estimated total intake of EDTA from all typical example fortified food-uses of Ferrazone® ferric sodium EDTA and all permitted food-uses of calcium disodium EDTA is summarised in Tables 2.7.3.3.4-1 and 2.7.3.3.4-2 on a mg/person/day and mg/kg body weight/day basis, respectively. Mean and 95th percentile all-user EDTA intakes from all typical example fortified food-uses of Ferrazone® ferric sodium EDTA and all permitted food-uses of calcium disodium EDTA were highest in male adults (28.72 mg/person/day) and female adults (68.31 mg/person/day), respectively. Children had the lowest all-user mean and 95th percentile intakes of EDTA on an absolute basis, at 12.90 and 27.51 mg/person/day, respectively.

Table 2.7.3.3.4-1 Summary of the Estimated Daily Intake of EDTA in the U.K. from All Typical Example Fortified Food-Uses of Ferrazone® Ferric Sodium EDTA and All Permitted Food-Uses of Calcium Disodium EDTA in the E.U. by Population Group (NDNS Data)

Population Group	Age Group (Years)	% User	Actual # of Total Users	All-Person Consumption			All-User Consumption		
				Mean (mg)	Percentile (mg)		Mean (mg)	Percentile (mg)	
					90	95		90	95
Children	1½ to 4½	98.2	1,619	12.86	22.63	27.51	12.90	22.60	27.51
Young People	4 to 10	99.5	833	18.30	31.08	36.38	18.34	31.24	36.38
Female Teenagers	11 to 18	97.5	435	20.31	33.21	44.67	20.47	33.47	45.41
Male Teenagers	11 to 18	99.3	413	23.47	40.72	48.70	23.44	40.51	48.19
Female Adults	16 to 64	93.6	897	25.02	41.60	68.00	25.44	42.64	68.31
Male Adults	16 to 64	94.1	721	28.16	50.44	64.65	28.72	50.91	64.77

On a body weight basis, children were again identified as having the highest intakes of any population group, with mean and 95th percentile all-user EDTA intakes of 0.90 and 1.85 mg/kg body weight/day, respectively. Male adults had the lowest mean and 95th percentile all-user intakes of EDTA from all typical example fortified food-uses of Ferrazone® ferric sodium EDTA and all permitted food-uses of calcium disodium EDTA on a body weight basis, at 0.35 and 0.82 mg/kg body weight/day, respectively.

Table 2.7.3.3.4-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of EDTA in the U.K. from All Typical Example Fortified Food-Uses of Ferrazone® Ferric Sodium EDTA and All Permitted Food-Uses of Calcium Disodium EDTA in the E.U. by Population Group (NDNS Data)

Population Group	Age Group (Years)	% User	Actual # of Total Users	All-Person Consumption			All-User Consumption		
				Mean (mg/kg bw)	Percentile (mg/kg bw)		Mean (mg/kg bw)	Percentile (mg/kg bw)	
					90	95		90	95
Children	1½ to 4½	98.2	1,619	0.89	1.56	1.85	0.90	1.57	1.85
Young People	4 to 10	99.5	833	0.71	1.24	1.47	0.72	1.25	1.47
Female Teenagers	11 to 18	97.5	435	0.38	0.71	0.87	0.39	0.71	0.87
Male Teenagers	11 to 18	99.3	413	0.43	0.77	0.91	0.44	0.78	0.91
Female Adults	16 to 64	93.6	897	0.35	0.65	0.97	0.38	0.67	1.04
Male Adults	16 to 64	94.1	721	0.33	0.62	0.79	0.35	0.64	0.82

2.7.3.3.4 Food Fortification Programs

In some, very specific instances, Ferrazone® ferric sodium EDTA, may be considered for use in food fortification programs. For example Romania is considering mandatory wheat flour fortification with micronutrients *e.g.,* iron and folic acid. There is a high incidence of anaemia

on the Romanian countryside. Therefore a short-term use of Ferrazone® ferric sodium EDTA could be proposed. For flour fortification we recommend 10 mg/kg Fe as Ferrazone® ferric sodium EDTA. As with any staple food (flour, rice, potatoes, *etc*.) the average daily consumption is 300 to 400 g. This means that 10 mg/kg would provide 3 to 4 mg/day Fe as Ferrazone® ferric sodium EDTA to the consumers, which should be enough to eliminate anaemia for around 80% in a population. This is a widely-accepted target percentage in food fortification policies.

2.7.4 Conclusions

Consumption data and information pertaining to the individual typical example fortified food-uses for Ferrazone® ferric sodium EDTA were used to estimate the all-person and all-user intakes of ferric sodium EDTA and iron of specific demographic groups in the U.K. population. This type of intake methodology is generally considered to be 'worst case' as a result of several conservative assumptions made in the consumption estimates. For example, it is often assumed that all food products within a food category contain the ingredient at the maximum specified level of use. In addition, it is well established that the length of a dietary survey affects the estimated consumption of individual users. Short-term surveys, such as the 4-day children's survey, may overestimate consumption of food products that are consumed relatively infrequently, particularly when weighted to 7 days (Gregory *et al*., 1995).

In summary, on an absolute basis, the greatest mean and 95th percentile all-user intakes of ferric sodium EDTA were determined in male adults (37.18 mg/person/day or 0.45 mg/kg body weight/day) and female adults (100.15 mg/person/day or 1.49 mg/kg body weight/day), respectively. These values correspond to mean (4.93 mg/person/day or 0.06 mg/kg body weight/day) and 95th percentile (13.28 mg/person/day or 0.20 mg/kg body weight/day) intakes of iron by male and female adults, respectively, from all typical example fortified food-uses of Ferrazone® ferric sodium EDTA in the E.U. In contrast, on an absolute basis, children had the lowest mean and 95th percentile all-user intakes of ferric sodium EDTA from all typical example fortified food-uses, with 16.63 and 35.97 mg/person/day (1.16 and 2.47 mg/kg body weight/day), respectively, which correspond to iron intakes of 2.21 and 4.77 mg/person/day (0.15 and 0.33 mg/kg body weight/day), respectively. These consumption estimates are far less than the estimated intake of 44 mg/person/day of iron from food, water, and supplements, as reported by the EVM for the U.K. (UK EVM, 2003). In addition, these levels comply with E.U. regulations and scientific opinions.

For EDTA, on an absolute basis, male adults also consumed the highest mean all-user intake of 25.45 mg/person/day (0.31 mg/kg body weight/day), while female adults had the highest 95th percentile all-user intake of 68.54 mg/person/day (1.02 mg/kg body weight/day) from all typical example fortified food-uses of Ferrazone® ferric sodium EDTA in the E.U. On a body weight basis, children consumed the greatest amount of EDTA from all typical example fortified food-uses of Ferrazone® ferric sodium EDTA in the E.U., with mean and 95th percentile all-user intakes of 0.79 and 1.69 mg/kg body weight/day, respectively. With the addition of all permitted food-uses of calcium disodium EDTA in the E.U., the mean and

95th percentile all-user intakes of EDTA by male and female adults increased to 28.72 and 68.31 mg/person/day (0.35 and 1.04 mg/kg body weight/day), respectively. In children, mean and 95th percentile all-user intakes of EDTA were 12.90 and 27.51 mg/person/day (0.90 and 1.85 mg/kg body weight/day), respectively, from all typical example fortified food-uses of Ferrazone® ferric sodium EDTA and all permitted food-uses of calcium disodium EDTA in the E.U. These consumption estimates are less than the ADI of 1.9 mg/kg body weight/day for EDTA re-established by JECFA (2007).

Ferrazone® ferric sodium EDTA has also been proposed for specific short-term use in food fortification programs at 10 mg/kg which would provide 3 to 4 mg/d Fe as Ferrazone® ferric sodium EDTA to the consumers.

2.8 Information on Existing Authorisations and Evaluations

Akzo Nobel Chemicals' Ferrazone® ferric sodium EDTA was determined to be Generally Recognized as Safe (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 {see Appendix E for Agency Response Letter GRAS Notice No. GRN 000178 [Sodium iron (III) ethylenediaminetetraacetate]} (FDA, 2006). In December 2004, sodium iron (III) ethylenediaminetetraacetate ($NaFe^{+3}EDTA$), as manufactured according to the procedure described by Kraft Foods Global, was considered to be GRAS for use as a source of dietary iron for food fortification purposes in powdered meal replacement, flavoured milk, and fruit-flavoured beverages at a use-level not to exceed 2.5 mg of iron per 200 mL of reconstituted beverage (GRAS Notice No. GRN 000152) (FDA, 2004).

In 1992, JECFA provisionally approved the use of ferric sodium EDTA in supervised food fortification programs, under conditions that additional studies be conducted, assessing the site of deposition of iron administered as ferric sodium EDTA and the metabolic fate of ferric sodium EDTA (JECFA, 1993). JECFA has since re-evaluated ferric sodium EDTA in 1999, at which time it removed the provisional conditions of approval and stated that the use of ferric sodium EDTA as a nutritional supplement in foods is considered acceptable when used in supervised food fortification programs that would provide iron intakes of approximately 0.2 mg/kg body weight/day from fortified foods (JECFA, 2000). This restriction of use in supervised fortification programs resulted from the mandate received from CCFAC who requested an evaluation for this specific purpose.

In 2007, JECFA re-evaluated ferric sodium EDTA and concluded as follows:

"Sodium iron EDTA is suitable for use as a source of iron for food fortification to fulfil nutritional iron requirements, provided that the total intake of iron from all food sources including contaminants does not exceed the PMTDI of 0.8 mg/kg body weight. Total intake of EDTA should not exceed acceptable levels, also taking into account the intake of EDTA from the food additive use of other EDTA compounds. An ADI of 0 to 2.5 mg/kg body weight was previously established for the calcium disodium and disodium salts of EDTA, equivalent to up to 1.9 mg/kg body weight EDTA." (JECFA, 2007). To summarise, JECFA noted that

the use of sodium iron EDTA should now be restricted only on the basis of not exceeding the ADI for EDTA *per se*.

In the recently issued "Guidelines on food fortification with micronutrients" by FAO/WHO ferric sodium EDTA has been explicitly highlighted as iron compound of choice for the "mass fortification of high-phytate cereal flours and for sauces with a high peptide content (e.g. fish sauce, soy sauce)" (FAO, 2006).

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/person/day, which would provide approximately 205 mg/ person/day of iron (Sweetman, 2002). Ferric sodium EDTA is used as an active pharmaceutical ingredient in Ferrostrane® (France), Sytron® (U.K.), and Ferritamin® (Sweden). Based on market information obtained by Akzo Nobel Functional Chemicals in 2001, Ferrostrane® has been registered as a medicine in Algeria, Benin, Burkina Faso, Cameroon, Congo, Gabon, Ghana, Guinea, Côte d'Ivoire, Madagascar, Mali, Mauritania, Mauritius, Morocco, Senegal, Togo, Tunisia and Vietnam. Akzo Nobel Functional Chemicals has supplied the active material for Ferrostrane® since 1986, resulting in the delivery of more than 200 tons of ferric sodium EDTA or 40 million 125 mL bottles of Ferrostrane®, without reported adverse effects.

Currently, the use of ferric sodium EDTA as a source of iron in foods and food supplements in the E.U. is not permitted. However, a comparable EDTA salt, calcium disodium EDTA (E385), is currently approved for use as an additive to such foods as minarine, 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 of the European Parliament and the Council of 20 February 1995 on food additives other than colours and sweeteners)* (European Parliament and The Council of the European Union, 1995). The use of calcium disodium EDTA and disodium EDTA as direct additives to foods is permitted in various jurisdictions around the world, including North and South America (*e.g.*, U.S.), Asia (*e.g.*, Malaysia, Philippines), Africa, and Australia (INACG, 1993; CFR, 2005b).

3.0 BIOLOGICAL AND TOXICOLOGICAL DATA

3.1 Bioavailability of Iron from Ferric Sodium EDTA Following Oral Consumption

Ferric sodium EDTA, which is highly soluble at physiological pH, has a persistently high bioavailability in foods despite the presence of inhibitory factors that form insoluble complexes with iron by hydrolytic polymerization (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). Iron in the form of ferric sodium EDTA is 2 to 3 times as bioavailable as iron in the form of $FeSO_4$ (Viteri *et al*., 1978; Martínez-Torres *et al*., 1979; MacPhail *et al*., 1981; Hurrell *et al*., 2000; Bothwell and MacPhail, 2004), and it is efficiently incorporated into

haemoglobin (MacPhail *et al*., 1981). The absorption of iron from ferric sodium EDTA is regulated physiologically by the body's iron status, in a manner similar to other iron compounds. Iron absorption is higher in subjects with low serum ferritin values compared to those with high serum ferritin concentrations (Candela *et al*., 1984; Hallberg *et al*., 1997; Hurrell *et al*., 2000; Mendoza *et al*., 2004). Additionally, increasing the level of iron added to wheat-soybean infant cereal from 5 to 15 mg was reported to result in a decreased percentage of iron absorption from ferric sodium EDTA in healthy male adults (Hurrell *et al*., 2000). Therefore, 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 (Hallberg *et al*., 1997; Yeung *et al*., 2004). Dietary iron fortification with ferric sodium EDTA is, thus, not expected to result in iron overload in iron-repleted individuals. A summary of the clinical bioavailability studies on ferric sodium EDTA is presented in Table 3.1-1.

Table 3.1-1 Summary of Human Bioavailability Studies on Ferric Sodium EDTA

Reference	Study Population	Carrier Food	Duration	Fe Exposure	Measured Endpoints	Results
Viteri *et al*. (1978)	7 healthy pre-school children (4 were slightly anaemic and iron-deficient)	250 mL of milk-rice-sugar formula fortified with FeNaEDTA, $Fe_2(SO_4)_3$, or Hb.	32 days	2.5 mg Fe from fortificant plus 2.5 mg Fe present intrinsically in the formula (5.0 mg Fe/day)	Fe absorption compared with a reference dose of ferrous ascorbate on Days 1, 2, 16, 17, and 32 of the study.	Absorption of Fe ?'d from FeNaEDTA (8.6%) compared to $Fe_2(SO_4)_3$ (3.3%), but ?'d compared to reference dose (76.2%) and Hb (34.5%).
	98 healthy and 21 iron-deficient and/or anaemic male adults from Guatemala (age not specified)	200 mL aqueous solution; Standard meal (consisting of 120 g black bean gruel, 120 g corn tortillas, 40 g bread, and 240 mL coffee) fortified with FeNaEDTA, $Fe_2(SO_4)_3$, or Hb.	1 day	5.0 mg Fe in aqueous solution; 0.4 mg Fe from fortificant plus 4.6 mg Fe present intrinsically in the meal	Fe absorption compared with a reference dose of ferrous ascorbate (time of measurement not specified).	Fe absorption from solution or meal was similar between FeNaEDTA (4.8-5.4%) and $Fe_2(SO_4)_3$ (2.6-5.0%), but was ?'d compared to reference dose (18.4-33.4%) and Hb (16.1-35.9%).
Martínez-Torres *et al*. (1979)	37 male and 80 female volunteers from Venezuela (age not specified)	10 g refined sugar, or 10 or 30 g of sugar cane syrup fortified with Fe(III)EDTA or $FeSO_4$ consumed alone or with wheat, sweet manioc, or maize meals.	2 days	3 or 5 mg Fe from fortificant	Absorption of Fe from Fe(III)EDTA or $FeSO_4$ measured 15 days after supplementation.	Similar Fe absorption from Fe(III)EDTA in sugar (8.4%) and sugar cane syrup (8.4%). Fe absorption from $FeSO_4$ in sugar (30.0%) and sugar cane syrup (25.8%) ?'d compared to Fe(III)EDTA. Consumption of wheat, sweet manioc, or maize did not affect Fe absorption from Fe(III)EDTA-fortified sugar cane syrup.

Table 3.1-1 Summary of Human Bioavailability Studies on Ferric Sodium EDTA						
Reference	**Study Population**	**Carrier Food**	**Duration**	**Fe Exposure**	**Measured Endpoints**	**Results**
		Wheat dough or sweet manioc flour fortified with Fe(III)EDTA or $FeSO_4$.	1 day	3 mg Fe from fortificant (1.4 mg Fe/100 g).	Fe absorption compared between the 2 sources and against a reference dose of ferrous ascorbate (time of measurement not specified).	?'d Fe absorption from wheat fortified with Fe(III)EDTA (11.5%) compared to $FeSO_4$ (4.9%). Similar Fe absorption from manioc fortified with Fe(III)EDTA (12.8%) and $FeSO_4$ (11.8%). Relative to reference dose, Fe absorption from foods fortified with Fe(III)EDTA remained stable (8-13%) compared to that from $FeSO_4$ (5-30%).
MacPhail *et al*. (1981)	5 separate studies conducted in a total of153 multiparous Indian women, aged 21 to 71 years	Water or maize porridge fortified with Fe(III)EDTA or $FeSO_4$ consumed alone or with bran, black tea, or 25, 50, or 100 mg ascorbic acid.	1 or 2 days	5 mg Fe from fortificant	Fe absorption compared between the 2 sources and against a reference dose of ferrous ascorbate (time of measurement not specified).	?'d Fe absorption from maize porridge fortified with Fe(III)EDTA compared to $FeSO_4$. Fe absorption from Fe(III)EDTA-fortified water was not affected by intake of bran, but ?'d by black tea. Absorption of Fe from Fe(III)EDTA enhanced by ascorbic acid only in the absence of inhibitors (*e.g.*, in porridge) and at sufficiently high concentrations (=50 mg).
Morck *et al*. (1983)	37 healthy volunteers, aged 18 to 50 years	200 mL water or coffee fortified with FeNaEDTA or $FeCl_3$.	2 days	3 mg Fe from fortificant	Absorption of Fe from Fe(III)EDTA or $FeCl_3$ measured 15 days after administration.	Similar Fe absorption from water or coffee fortified with Fe(III)EDTA or $FeCl_3$. Relative Fe absorption from Fe(III)EDTA or $FeCl_3$ was constant regardless of the beverage.

Table 3.1-1 Summary of Human Bioavailability Studies on Ferric Sodium EDTA

Reference	Study Population	Carrier Food	Duration	Fe Exposure	Measured Endpoints	Results
Hurrell *et al*. (2000)	10 male and 19 female healthy volunteers, aged 22 to 27 years	Wheat, wheat-soy, or Quinoa infant cereal fortified with FeNaEDTA, ferrous fumarate, or $FeSO_4$.	31 days	5 mg of total Fe	Fe absorption compared between fortificants (time of measurement not specified).	Fe absorption from cereals fortified with FeNaEDTA (1.7-5.2%) 2- to 4-fold higher than with $FeSO_4$ (0.6-2.2%) or ferrous fumarate (0.6-2.1%).
	4 male and 5 female healthy volunteers, mean age 23 years	Wheat-soybean infant cereal fortified with FeNaEDTA or $FeSO_4$.		5 or 15 mg of total Fe		Fe absorption ?'d from FeNaEDTA compared to $FeSO_4$. Increasing Fe intake from 5 to 15 mg slightly ?'d percentage of Fe absorption from both sources.
	11 male and 9 female healthy volunteers, aged 25 or 26 years	Low-extraction-wheat (LEW) or high-extraction-wheat (HEW) bread rolls fortified with FeNaEDTA or $FeSO_4$.		5 mg of total Fe		Absorption of Fe from LEW or HEW rolls fortified with FeNaEDTA (3.9-11.5%) ?'d compared to $FeSO_4$ (1.0-5.7%).
Layrisse *et al*. (2000)	5 male and 21 female Venezuelan volunteers of low socio-economic status, aged 15 to 50 years	Bread prepared from precooked corn flour or white wheat flour fortified with Fe(III)EDTA, $FeSO_4$, or iron bisglycine chelate (Ferrochel).	4 meals	3 mg Fe from fortificant	Absorption of Fe from Fe(III)EDTA, $FeSO_4$, and Ferrochel measured 15 days after consumption.	Fe absorption from bread fortified with Ferrochel (8.4-10.8%) and Fe(III)EDTA (10.5-14.9%) ?'d compared with $FeSO_4$ (4.7-5.3%).
Walter *et al*. (2003)	45 healthy women (age not specified)	Tortillas prepared from corn masa flour fortified with FeNaEDTA or ferrous bisglycinate.	3 meals	30 mg Fe/kg flour from fortificant plus 15 mg Fe/kg flour present intrinsically in the flour	Fe absorption compared between the fortificants (time of measurement not specified).	Absorption of Fe ?'d from FeNaEDTA compared to ferrous bisclycinate.
Davidsson *et al*. (2002)	33 healthy females from Guatemala, aged 12 or 13 years	Meal consisting of corn tortillas, 50 g black bean paste, and 250 g deionised water fortified with FeNaEDTA, ferrous fumarate, or $FeSO_4$.	2 meals per day for 2 days	2 mg Fe from fortificant plus 2.2 mg Fe present intrinsically in the meal	Absorption of Fe from FeNaEDTA, ferrous fumarate, and $FeSO_4$ measured 14 days after consumption.	Bioavailability of Fe ?'d from FeNaEDTA (9.0%) compared to ferrous fumarate (6.2%) or $FeSO_4$ (5.5%).

Table 3.1-1 Summary of Human Bioavailability Studies on Ferric Sodium EDTA

Reference	Study Population	Carrier Food	Duration	Fe Exposure	Measured Endpoints	Results
Fidler *et al*. (2003)	50 healthy adult females, aged 19 to 29 years (16 of whom were iron-deficient, while 2 had iron-deficiency anaemia)	Meals consisting of rice or rice and vegetables with 10 g of fish sauce or soy sauce fortified with FeNaEDTA or $FeSO_4$.	2 days	5 mg Fe from fortificant plus 0.1-0.9 mg Fe present intrinsically in the meal	Absorption of Fe from FeNaEDTA and $FeSO_4$ measured 14 days after meal consumption.	Similar Fe absorption from fish sauce and soy sauce fortified with FeNaEDTA (3.3-6.1%) or $FeSO_4$ (3.1-5.6%). Fe absorption from meals not affected by addition of fish sauce, but ?'d by addition of soy sauce.
Davidsson *et al*. (2005)	5 male and 6 female infants, aged 18 to 27 weeks (2 were anaemic, and 1 had low plasma ferritin levels)	Wheat or soy-based infant cereal fortified with FeNaEDTA or $FeSO_4$ plus ascorbic acid.	2 meals in 1 day	2 mg Fe from fortificant plus 2 mg Fe/100 g cereal present intrinsically	Fe absorption compared between fortificants 14 days after consumption.	Similar Fe absorption from infant cereal fortified with FeNaEDTA (3.7%) and $FeSO_4$ plus ascorbic acid (4.9%).
Mendoza *et al*. (2001)	14 healthy, non-pregnant, adult women, aged 19 to 42 years	Porridge prepared from low-phytate maize (LPM) or wild-type maize (WTM) fortified with FeNaEDTA or $FeSO_4$.	2 meals for 2 days	Total Fe of 4.4 mg, with 1 mg Fe/serving from fortificant	Fe absorption compared between fortificants 10 days after consumption.	Bioavailability of Fe ?'d from FeNaEDTA (5.4-5.7%) compared to $FeSO_4$ (1.7-1.9%) in both LPM and WTM porridges.
Mendoza *et al*. (2004)	13 healthy, non-pregnant, adult women, aged 20 to 31 years	Foods containing a fortification mixture of FeNaEDTA or $FeSO_4$, 192 or 200 mg Ca^{2+} as $Ca_3(PO_4)_2$, 10 mg Zn^+ as zinc methionine or $ZnSO_4$, 50 or 100 mg ascorbic acid, and 1 g citric acid.	7 days	10 mg Fe from fortificant plus 4.7 mg Fe present intrinsically in the food product	Fe incorporation into red blood cells compared between sources, and determined on Days 1, 15, 22, and 29 of the study.	Fe absorption from FeNaEDTA ?'d by 1.7-fold compared to $FeSO_4$.

Table 3.1-1 Summary of Human Bioavailability Studies on Ferric Sodium EDTA						
Reference	**Study Population**	**Carrier Food**	**Duration**	**Fe Exposure**	**Measured Endpoints**	**Results**
Andang'o *et al.* (2007)	516 children, aged 3 to 8 years	The porridge made from fortified whole maize flour	5 months	The porridge for one group was made from unfortified whole maize flour; for the other three groups it was fortified with either high-dose ferric sodium EDTA (56 mg/kg), low-dose ferric sodium EDTA (28 mg/kg), or electrolytic iron (56 mg/kg).	Haemoglobin, plasma ferritin, and transferrin receptor. The primary outcome was iron-deficiency anaemia.	The results showed that the prevalence of iron-deficiency anaemia in children given unfortified flour was 10%. Compared with placebo, the prevalence of iron-deficiency anaemia in children given flour fortified with high-dose ferric sodium EDTA, low-dose ferric sodium EDTA, and electrolytic iron changed by -89% (95% CI -97% to -49%), -48% (-77% to 20%), and 59% (-18% to 209%), respectively. Consumption of high-dose ferric sodium EDTA improved all measured iron-status indicators. Low-dose ferric sodium EDTA decreased the prevalence of iron deficiency but did not noticeably change the prevalence of anaemia.

3.1.1 Human Studies

Viteri *et al.* (1978) conducted a series of studies to compare the absorption of iron from ferric sodium EDTA, ferric sulphate [$Fe_2(SO_4)_3$], and haemoglobin in healthy or iron-deficient children and adults. In all studies, ferrous ascorbate (providing 5 mg iron) was given to the subjects as a reference compound. All iron compounds were biosynthetically labelled with ^{59}Fe or ^{55}Fe. The results of the studies are briefly summarised in Table 3.1.1-1. Seven (7) healthy pre-school children were given a single feeding of 250 mL of a milk-rice-sugar formula containing 2.5 mg of iron as ferric sodium EDTA, $Fe_2(SO_4)_3$, or haemoglobin. The total amount of iron administered to the children was 5 mg, with 2.5 mg of iron occurring naturally in the diet. Absorption tests were conducted on Days 1, 2, 16, and 17 of the study, while blood samples were collected on Days 16 and 32. It was reported that 4/7 (57%) of the children were slightly anaemic and iron-deficient, as evidenced by a greater than 60% reference dose absorption. Mean absorption of iron from haemoglobin was similar to that from ferrous ascorbate, but was significantly higher that that from ferric sodium EDTA or $Fe_2(SO_4)_3$. However, mean iron absorption from ferric sodium EDTA was significantly higher than that from $Fe_2(SO_4)_3$.

Ninety-eight (98) healthy male adults received 5.0 mg of total iron from ferric sodium EDTA, $Fe_2(SO_4)_3$, or haemoglobin in the form of an aqueous solution or a standard meal. The standard meal, which consisted of black bean gruel (120 g), 4 corn tortillas (120 g), a roll of bread (40 g), and a cup of coffee, was fortified with 0.4 mg of iron from ferric sodium EDTA, $Fe_2(SO_4)_3$, or haemoglobin, with the remaining 4.6 mg of iron occurring naturally in the meal. Ferric sodium EDTA and $Fe_2(SO_4)_3$, labelled with different iron isotopes, were mixed together in the same meal and heated prior to serving. Iron absorption from haemoglobin was similar to that from ferrous ascorbate, and significantly higher that that from ferric sodium EDTA and $Fe_2(SO_4)_3$. Absorption of iron from ferric sodium EDTA was not significantly different from $Fe_2(SO_4)_3$, regardless of whether the iron was administered in aqueous solution or as part of a meal. Iron-deficient subjects were fed a similar meal as that consumed by healthy subjects; however, ferric sodium EDTA and $Fe_2(SO_4)_3$ were mixed separately into the diet on different days. Iron absorption from ferric sodium EDTA in iron-deficient subjects was significantly increased (2.7-fold) compared to that from $Fe_2(SO_4)_3$, while iron absorption from haemoglobin was significantly higher than that from ferric sodium EDTA or $Fe_2(SO_4)_3$. Out of 11 anaemic subjects, only 3 were reported to have an iron absorption value of greater than 4.5% and less than 10% from $Fe_2(SO_4)_3$, while 10 were reported to have iron absorption values of up to 19.9% from ferric sodium EDTA.

The effect of the addition of an inhibitor (desferrioxamine) and an enhancer (ascorbic acid) of iron absorption also was investigated by Viteri *et al.* (1978). The addition of 500 mg of desferrioxamine to the iron-fortified meals of healthy adults reduced the percentage of iron absorption from both ferric sodium EDTA and $Fe_2(SO_4)_3$; however, iron absorption from ferric sodium EDTA remained significantly higher than that from $Fe_2(SO_4)_3$. The authors attributed this to the fact that ferric sodium EDTA remains highly soluble at normal pH, which impairs the formation of complexes with inhibitors, such as desferrioxamine. Ingestion of a drink containing 500 mg of ascorbic acid together with the iron-fortified meals enhanced the

absorption of iron from both ferric sodium EDTA and $Fe_2(SO_4)_3$, with iron absorption from ferric sodium EDTA being slightly higher than from $Fe_2(SO_4)_3$.

Table 3.1.1-1 Absorption of Iron from $Fe_2(SO_4)_3$, Ferric Sodium EDTA, Haemoglobin and Ferrous Ascorbate

Subject	Study Design	Iron Absorption[a] (%)			
		$Fe_2(SO_4)_3$	Ferric Sodium EDTA	Haemoglobin	Ferrous Ascorbate[b]
Pre-school children	$Fe_2(SO_4)_3$, FeNaEDTA, or haemoglobin mixed with a milk-rice-sugar formula[e]	3.3[c]	8.6[c]	34.5	76.2
Healthy male adults	$Fe_2(SO_4)_3$, FeNaEDTA, or haemoglobin given alone, in a fasting state	2.6[g]	4.8[g]	16.1[d]	18.4
Healthy male adults	$Fe_2(SO_4)_3$, FeNaEDTA, or haemoglobin mixed with a breakfast meal[f]	5.0[g]	5.4[g]	35.9	33.4
Rural male adults	$Fe_2(SO_4)_3$, FeNaEDTA, or haemoglobin mixed with a breakfast meal	2.4[h]	6.4[h]	--	48.2
Healthy male adults	$Fe_2(SO_4)_3$, FeNaEDTA, or haemoglobin mixed with a breakfast meal	0.6[h] (+D)[i] 6.8 (+AA)[i]	1.8[h] (+D) 9.5 (+AA)	--	--

Adapted from Viteri *et al*., 1978
[a] Values expressed as geometric means.
[b] Ferrous ascorbate administered in a solution containing 500 mg of ascorbic acid; used as the reference dose.
[c] Significantly different from ferrous ascorbate, $p < 0.02$.
[d] Administered with 200 mL of Pepsi Cola.
[e] Formula containing 16.5 g of a rice-milk-sugar mixture (60:16:24), plus vitamins and $FePO_4$.
[f] Meal consisting of black bean gruel (120 g), 4 corn tortillas (120 g), a roll of bread (40 g) and a cup of coffee.
[g] Statistical significance not reported.
[h] Significantly different from ferrous ascorbate, $p < 0.001$.
[i] Administered with: D – desferrioxamine, AA – ascorbic acid.

The absorption of 3 or 5 mg of iron from $FeSO_4$ or Fe(III)EDTA was studied in 107 volunteers (37 males and 80 females) from the rural areas of Venezuela, using 10 g of refined sugar and 10 or 30 g of sugar cane syrup as vehicles (Martínez-Torres *et al*., 1979). The results of the absorption studies are briefly summarised in Table 3.1.1-2. The absorption of iron following the consumption of fortified sugar or sugar cane syrup alone, or the consumption of sugar cane syrup in combination with food products made of wheat, manioc, or maize was assessed. The subjects were provided with meals (radioactively labelled with ^{55}Fe or ^{59}Fe) on 2 consecutive mornings following an overnight fast, and blood samples were obtained 15 days after the first administration of the labelled meal. On Days 15 and 16, the subjects received meals labelled with the alternative radioisotope tracer, and their blood samples were obtained on Day 30. There was no significant difference in iron absorption from Fe(III)EDTA-fortified refined sugar or sugar cane syrup; however, iron absorption from 30 g of syrup was significantly higher than that from 10 g of syrup. Absorption of iron from $FeSO_4$-fortified syrup was significantly higher than that from Fe(III)EDTA. When sugar cane syrup fortified with Fe(III)EDTA was mixed with various food preparations, there was no significant difference in iron absorption following the consumption

of wheat-, sweet manioc-, or maize-based products. In another experiment, the absorption of intrinsic and extrinsic iron in fortified wheat was determined. Biosynthetically labelled wheat (containing 3 mg of iron) was mixed with 3 mg of iron from labelled Fe(III)EDTA or $FeSO_4$ in 2 different meals. Mean iron absorption from intrinsic and extrinsic iron was not significantly different; however, the absorption of iron from Fe(III)EDTA was greater than that from $FeSO_4$. According to the authors, iron from Fe(III)EDTA exchanges completely with vegetal iron in the lumen of the gut, such that intrinsic and extrinsic iron absorption are relatively the same. In the final experiment, 3 mg of iron from Fe(III)EDTA or $FeSO_4$ was mixed with wheat dough or sweet manioc flour (containing 1.4 mg iron/100 g). Absorption of iron from Fe(III)EDTA was significantly greater than that from $FeSO_4$ following consumption of wheat flour; however, there was no significant difference in absorption between the 2 iron sources following consumption of sweet manioc flour. Iron absorption from Fe(III)EDTA-fortified foods remained practically the same, ranging from 8 to 13% of the reference dose absorption (iron ascorbate), while absorption from $FeSO_4$-fortified foods varied widely, ranging from 5 to 30% of the reference dose absorption. However, mean iron absorption values were not significantly different from each other. The authors suggested that iron absorption from $FeSO_4$ was very sensitive to absorption inhibiting substances present in food vehicles, while absorption from Fe(III)EDTA is only slightly affected.

Table 3.1.1-2 Reported Absorption Values* of Iron from Fe(III)EDTA and $FeSO_4$ Mixed with Various Foods

Fortified Food	Iron Absorption (%)		
	Fe(III)EDTA (3 or 5 mg of iron)	$FeSO_4$ (3 or 5 mg of iron)	Composite Mean Absorption from Reference Dose (3 mg of iron)
Refined sugar	8.4	30.0	31.5
Sugar cane syrup	8.4	25.8	
Sweet manioc	12.8	11.8	
Wheat	11.5	4.9	

Martínez-Torres *et al*., 1979
* Calibrated according to the absorption from the reference dose.

The absorption of 5 mg of iron (approximately 0.083 mg iron/kg body weight) as Fe(III)EDTA or $FeSO_4$ was examined in 5 separate studies involving 153 multiparous Indian women (aged 21 to 71 years) (MacPhail *et al*., 1981). Pre-intervention values of the different evaluated parameters were obtained from all of the subjects, and each subject served as his or her own control. In the first study, the absorption of 5 mg iron from $FeSO_4$- or Fe(III)EDTA-fortified maize porridge was examined in 12 subjects. The subjects received a $FeSO_4$-fortified meal on one morning, and a Fe(III)EDTA-fortified meal the next morning. Mean iron absorption values from the Fe(III)EDTA-fortified porridge were significantly greater than from the $FeSO_4$-fortified meal. Additionally, iron absorption values were significantly decreased in subjects given $FeSO_4$-fortified water with bran, while no significant change in iron absorption was noted in subjects given Fe(III)EDTA-fortified water and bran relative to

baseline. Ingestion of black tea, however, significantly reduced iron absorption from Fe(III)EDTA-fortified water by 7-fold.

In another experiment, the effect of administering increasing doses of ascorbic acid was studied in 6 groups of subjects (9 to 12/group) (MacPhail *et al.*, 1981). On the first day, subjects were provided with water or maize porridge fortified with 5 mg of iron as Fe(III)EDTA, together with 25, 50, or 100 mg of ascorbic acid. The following day, subjects received Fe(III)EDTA alone in water or maize porridge. After the subjects had completed each of the 2-day set of experiments, the absorption of 3 mg of iron as ferrous ascorbate was measured, and iron absorption results were standardized to 40% of the reference absorption value. When compared to non-standardized iron absorption values obtained following ingestion of Fe(III)EDTA alone in water or maize porridge, significantly increased values were observed following consumption of Fe(III)EDTA in water and 50 or 100 mg of ascorbic acid, as well as after ingestion of Fe(III)EDTA in porridge and 100 mg of ascorbic acid. Standardized iron absorption values from Fe(III)EDTA-fortified maize porridge were significantly lower than those obtained from Fe(III)EDTA-fortified water and 100 mg ascorbic acid. Based on the results of the study, the authors concluded that the absorption of iron from Fe(III)EDTA is inhibited by tea, but not by bran or maize porridge. Furthermore, iron absorption is enhanced by ascorbic acid only in the absence of inhibitors, and sufficiently high concentrations of ascorbic acid (molar ascorbate to iron ratio of 6:1) are required to enhance the absorption of iron from Fe(III)EDTA.

Table 3.1.1-3 The Reported Effects of Inhibitors on the Absorption of Iron from $FeSO_4$ and Fe(III)EDTA Administered in 100 mL Water

Inhibitor	Iron Source	No. of Subjects	Mean Haemoglobin (g/L)	Serum Ferritin* (µg/L)	Fe absorption (%)*[#]		
					Reference[t] Salt	Without Inhibitor	With Inhibitor
10 g Bran	$FeSO_4 \cdot 7H_2O$	9	137	33.0	22.5	16.5	1.5[b]
10 g Bran	Fe(III)EDTA	10	120	10.7	43.3	10.3	8.4[a]
Tea[T]	Fe(III)EDTA	8	129	21.1	49.2	19.2	2.8[b]

MacPhail *et al.*, 1981
* Geometric means used because values were positively skewed.
[t] 3 mg of iron as ferrous ascorbate given in a fasting state.
[T] 45 g tea leaves added to 1,800 mL boiling water to give 200 mg/subject.
[#] Absorption values (%) were calculated under the assumption that 100% of the absorbed radioactivity was present in the haemoglobin, and that each subject's blood volume was 65 mL/kg.
[a] Not significant, $p > 0.1$ compared to without inhibitor.
[b] Significant, $p < 0.001$ compared to without inhibitor.

Table 3.1.1-4 Effects of Ascorbic Acid (AA) on the Absorption of Iron from Fe(III)EDTA Administered in 100 mL Water or 250 g Maize Porridge

No. of Subjects	AA (mg/meal)	Fe:AA Molar Ratio	Mean Haemoglobin (g/L)	Serum* Ferritin (µg/L)	Fe Absorption (%)*#				
					Actual Absorption		Reference[t] Salt	Standardized[T] Absorption	
					Without AA	With AA	Mean	Without AA	With AA
Fe(III)EDTA in 100 mL Water									
11	25[d]	1:1.5	134	25	6.4	6.6[a]	37.4	7.2	8.0[a]
10	50[d]	1:3	133	31	7.7	11.0[b]	26.8	9.8	16.8[b]
9	100[d]	1:6	134	14	7.0	47.7[c]	41.5	6.8	44.0[c]
Fe(III)EDTA in 250 g Maize Porridge									
12	25[e]	1:1.5	142	19	6.4	6.9[a]	25.4	8.5	8.9[a]
11	50[e]	1:3	137	33	6.3	6.0[a]	20.4	12.0	12.0[a]
9	100[e]	1:6	138	21	6.1	12.0[c]	30.5	8.0	15.6[b]

MacPhail *et al*., 1981
* Geometric means used because values were positively skewed.
[t] 3 mg of iron as ferrous ascorbate given in a fasting state.
[T] Standardized to 40% of reference dose absorption.
[#] Absorption values (%) were calculated under the assumption that 100% of the absorbed radioactivity was present in the haemoglobin, and that each subject's blood volume was 65 mL/kg.
[a] Not significant, $p > 0.1$ compared to without AA.
[b] Significant, $p < 0.05$ compared to without AA.
[c] Significant, $p < 0.01$ compared to without AA.
[d] Fe(III)EDTA given in 100 mL water.
[e] Fe(III)EDTA given in 250 g maize porridge.

Iron absorption studies were conducted in 37 healthy volunteers aged 18 to 50 years (Morck *et al*., 1983). In one of the studies, subjects were provided with 200 mL of water or coffee, containing 3 mg of iron (^{55}Fe or ^{59}Fe) as $FeCl_3 \cdot 6H_2O$ or ferric sodium EDTA. The subjects received $FeCl_3$-fortified water or coffee on consecutive days, while ferric sodium EDTA-fortified water or coffee was similarly administered 2 weeks later. Blood samples were obtained 2 weeks after the administration of each pair of beverages, and non-haem iron absorption was determined by measuring incorporated red cell radioactivity, assuming that erythrocyte incorporation represented 80% of the absorbed iron. Iron absorption was not significantly different between ferric sodium EDTA and $FeCl_3 \cdot 6H_2O$ in water, although absorption from ferric sodium EDTA (3.83%) was slightly lower than that from $FeCl_3$ (5.04%). A significant 70% reduction in iron absorption was observed from coffee fortified with ferric sodium EDTA or $FeCl_3 \cdot 6H_2O$ compared to that observed from fortified water; however, the relative percentage of iron absorption from ferric sodium EDTA or $FeCl_3 \cdot 6H_2O$ remained constant regardless of the beverage.

Eighty-four (84) healthy male and female volunteers, aged 18 to 40 years, (15 of whom had serum ferritin levels <12 µg/L) were placed into 9 different treatment groups (3 to 8/sex/group), wherein they received appropriate test meals for a period of 31 days (Hurrell *et al*., 2000). Brief summaries of the results are listed in Tables 3.1.1-5 and 3.1.1-6. For control, subjects were asked to consume unfortified test meals. In studies 1 to 3, subjects were fed infant cereals fortified with $Na^{55}FeEDTA$, [^{55}Fe]-ferrous fumarate, or $^{55}FeSO_4$. In

study 4, subjects were provided with wheat-soybean cereal fortified with $Na^{55}FeEDTA$ or $^{55}FeSO_4$. In study 5, subjects were given 2 low-extraction-wheat (LEW) rolls fortified with $Na^{55}FeEDTA$ or $^{55}FeSO_4$, with or without tea; while in study 6, subjects received 2 high-extraction-wheat (HEW) rolls unfortified or fortified with $Na^{55}FeEDTA$ or $^{55}FeSO_4$. In study 7, subjects were fed with 4 mg iron as $^{55}FeSO_4$-fortified wheat cereal; while in study 8, subjects were provided with 4 mg iron as $^{55}FeSO_4$-fortified wheat-soybean cereal. In study 9, subjects received wheat infant cereal fortified with 4 mg iron as $^{55}FeSO_4$ or [^{55}Fe]-ferric pyrophosphate. The meals were fortified with 16, 32, and 28% of iron with the use of $Na^{55}FeEDTA$, [^{55}Fe]-ferrous fumarate and [^{55}Fe]-ferric pyrophosphate, respectively, and mixed with the appropriate amounts of non-radioactive iron to provide 5 mg (studies 1 to 3) or 5 or 15 mg (study 4) of iron to each subject per meal.

Ferrous fumarate absorption was similar to $FeSO_4$ in studies 1, 2, and 3, with iron absorption ranging from 0.57 to 5.23% of the iron administered. In addition, absorption of iron from ferric sodium EDTA-fortified cereals was 2- to 4-fold higher than $FeSO_4$-fortified meals. In study 4, increasing iron intake levels from 5 to 15 mg/meal slightly decreased the percentage of iron absorption of subjects (from 3.32 to 1.94% for ferric sodium EDTA and 0.94 to 0.72% for $FeSO_4$); however, iron absorption from ferric sodium EDTA was significantly greater than $FeSO_4$ at both iron concentrations. Mean iron absorption was significantly greater in ferric sodium EDTA-fortified LEW rolls (11.5%) than $FeSO_4$-fortified HEW rolls (0.99%) in studies 5 and 6. According to the authors, ferric sodium EDTA enhanced, but did not completely reverse, the inhibition of iron absorption by phytate-containing foods [*e.g.*, iron absorption from phytate-free HEW rolls (3.91%) was still lower than from LEW rolls (11.5%)].

The inhibiting effects of polyphenols from tea also were not affected by ferric sodium EDTA fortification, as the administration of tea with the test meals significantly decreased iron absorption from both $FeSO_4$ and ferric sodium EDTA; however, iron absorption from ferric sodium EDTA-fortified cereals was still 2- to 4-fold higher than those fortified with $FeSO_4$. In study 9, iron absorption from ferric pyrophosphate (0.26%) was slightly lower, but not significantly different, from that of $FeSO_4$ (1.76%); however, the addition of disodium EDTA significantly increased iron absorption from $FeSO_4$ (5.93%), with a 13-fold increase over that of ferric pyrophosphate (0.44%). A similar effect was reported in study 7, following the addition of disodium EDTA to $FeSO_4$-fortified wheat cereal. Iron absorption increased by more than 5-fold, from 1.02% with $FeSO_4$ alone to 5.71% with $FeSO_4$ + disodium EDTA. In contrast, only a slight increase in iron absorption (from 0.70 to 2.86%) was reported in study 8, with the addition of disodium EDTA to $FeSO_4$-fortified wheat-soybean cereal. According to the authors, individual iron absorption is highest in those subjects with the lowest serum ferritin values. Furthermore, dietary iron absorption is inversely related to content, such that iron absorption is highest in unfortified foods and lowest in foods fortified with 15 mg of iron. According to Hallberg *et al*. (1997), it is evident that the body maintains iron levels in the body through certain down-regulating systems, which control the amount of iron absorbed and protects against the possibility of iron-overload.

Table 3.1.1-5 Reported Iron Absorption from Cereal-Based Foods Fortified with Ferric Sodium EDTA, $FeSO_4$ and Ferrous Fumarate in Adult Human Subjects

Study No.	Study Design	Mean Serum Ferritin (µg/L)	Test Meals	Mean Iron Absorption (% dose)	Mean Absorption Ratio
1	Wheat infant cereal (3 M, 5F, 22 years)	39	Ferrous fumarate	2.06	0.94
			Unfortified	3.90	1.78*
			FeNaEDTA	5.23	2.37***
			$FeSO_4$	2.20	-
2	Wheat-soy infant cereal (4 M, 5F, 27 years)	42	Ferrous fumarate	0.93	1.28
			Unfortified	1.13	1.56*
			FeNaEDTA	2.81	3.86***
			$FeSO_4$	0.73	-
3	Quinoa infant cereal (3 M, 4F, 22 years)	39	Ferrous fumarate	0.57	0.91
			Unfortified	0.67	1.07
			FeNaEDTA	1.68	2.60**
			$FeSO_4$	0.63	-
4	Wheat-soyabean infant cereal (4M, 5F, 23 years)	41	FeNaEDTA	3.32	3.53**
			$FeSO_4$	0.94	-
			FeNaEDTA x 3	1.94	2.70**
			$FeSO_4$ x 3	0.72	-
5	LEW bread roll (5M, 5F, 25 years)	54	FeNaEDTA	11.50	2.02**
			$FeSO_4$	5.70	-
			FeNaEDTA, tea	1.86	1.81**
			$FeSO_4$, tea	1.03	-
6	HEW bread roll (6M, 4F, 26 years)	20	Unfortified	2.27	2.29*
			FeNaEDTA	3.91	3.94***
			$FeSO_4$	0.99	-
9	Wheat infant cereal (3M, 8F, 24 years)	29	Ferric pyrophosphate	0.26	-
			$FeSO_4$	1.76	-
			Ferric pyrophosphate + Na_2EDTA	0.44	1.68
			$FeSO_4$ + Na_2EDTA	5.93	3.37***T

Hurrell *et al*., 2000
M – male; F – female; [T]Absorption ratios with and without Na_2EDTA
Mean values were significantly different from study 1: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.
Absorption from test meal compared with absorption from $FeSO_4$ (studies 1-6), $FeSO_4$ x 3 (study 4) or $FeSO_4$ plus tea (study 5) test meals.

Table 3.1.1-6 Reported Iron Absorption from Wheat Cereal-Based Foods Fortified with $FeSO_4$ in the Presence of Disodium EDTA in Adult Human Subjects

Study No.	Study Design	Mean Serum Ferritin (µg/L)	Test Meals (EDTA:iron molar ratios)	Mean Iron Absorption (% dose)
7	Wheat infant cereal (5 M, 5F, 25 years)	21	0:1.0	1.02
			0.33:1.0	2.53
			0.67:1.0	5.71
			1.0:1.0	5.59
8	Wheat-soybean infant cereal (3M, 7F, 24 years)	24	0:1.0	0.70
			0.33:1.0	1.76
			0.67:1.0	2.22
			1.0:1.0	2.86

Hurrell *et al*., 2000
M – male; F – female

Layrisse *et al*. (2000) conducted a study of comparing the bioavailability of iron from ferrous bisglycinate, Fe(III)EDTA, and $FeSO_4$. A total of 74 healthy volunteers, aged 15 to 50 years (18 males and 56 females) from a low socio-economic stratum of Valencia, Venezuela participated in 5 different absorption studies. Each subject was allowed to participate in only 1 out of 5 studies, and participation was determined by random selection. For each study, the number of participants and the meals administered are presented in Table 3.1.1-7. Fifteen (15) days after ingestion of the appropriate meals (*i.e.*, Study Days 16 and 30), blood samples were obtained from each of the subjects for measurement of erythrocyte radioactivity, haemoglobin and serum ferritin concentrations. In Study 1, iron absorption from foods fortified with ferrous bisglycinate (8.4%) or Fe(III)EDTA (10.5%) was significantly greater than that obtained from unfortified (3.2%) or $FeSO_4$-fortified foods (4.7%). The mean iron absorption of 4/13 subjects who were iron-deficient in Study 1 also was reported to be significantly greater from foods fortified with ferrous bisglycinate (13%) or Fe(III)EDTA (14%) compared to that obtained from $FeSO_4$-fortified foods (6%). In Study 2, iron absorption from foods fortified with ferrous bisglycinate (10.8%) or Fe(III)EDTA (14.9%) was significantly greater than that obtained from unfortified (3.0%) or $FeSO_4$-fortified foods (5.3%). The mean iron absorption of 4/13 subjects who were iron-deficient in Study 2 also was significantly higher from foods fortified with ferrous bisglycinate (12%) or Fe(III)EDTA (15%) compared to from foods fortified with $FeSO_4$ (7%).

Table 3.1.1-7 Composition of Meals Fortified with Ferrous Bisglycinate, $FeSO_4$, or Fe(III)EDTA Administered to Subjects Participating in Iron Absorption Studies[1]					
Study No.	**No. of Subjects**	**Meal A**	**Meal B**	**Meal C**	**Meal D**
1	4 males, 9 females	Basal corn flour meal[2] provided in the morning of Day 1	Basal corn flour meal + 3 mg of iron as $^{55}FeSO_4$ provided in the afternoon of Day 1	Basal corn flour meal + 3 mg of iron as ^{59}Fe-ferrous bisglycinate provided in the morning of Day 15	Basal corn flour meal + 3 mg of iron as ^{55}FeEDTA provided in the afternoon of Day 15
2	1 male, 12 females	Basal wheat flour meal[3] provided in the morning of Day 1	Basal wheat flour meal + 3 mg of iron as $^{55}FeSO_4$ provided in the afternoon of Day 1	Basal wheat flour meal + 3 mg of iron as ^{59}Fe-ferrous bisglycinate provided in the morning of Day 15	Basal wheat flour meal + 3 mg of iron as ^{55}FeEDTA provided in the afternoon of Day 15

[1] Table adapted from Layrisse *et al.* (2000).
[2] The basal corn flour meal consisted of 100 g pre-cooked corn flour + 50 g cheese + 10 g margarine, with a basal iron content of 1.5 mg.
[3] The basal wheat flour meal consisted of 100 g white wheat flour + 50 g cheese + 10 g margarine, with a basal iron content of 1.6 mg.

Walter *et al.* (2003) conducted another bioavailability study in which 3 groups of healthy women (15/group) consumed diets consisting of tortillas made from corn masa flour. The corn masa flour, which contained 15 mg of native iron/kg flour, was supplemented with 30 mg of iron as ferrous fumarate, ferrous bisglycinate, or ferric sodium EDTA/kg flour. Each of the groups was given 3 test meals and a reference dose (3 mg ferrous ascorbate). A total of 6 meals were tested: tortillas made from unfortified corn masa flour (Meal 1; 15 mg total iron/kg flour); tortillas from unfortified flour + black beans, rice, and chilli sauce, contributing 2 mg non-haem iron/kg + disodium EDTA (Meal 2; 17 mg total iron/kg); tortillas from flour fortified with ferrous fumarate, in the absence and presence of disodium EDTA (Meals 3 and 4, respectively; 45 mg total iron/kg flour); tortillas from flour fortified with ferrous bisglycinate (Meal 5; 45 mg total iron/kg flour); and tortillas from flour fortified with ferric sodium EDTA (Meal 6; 45 mg total iron/kg flour). Absorption of iron from meals that were not supplemented with disodium EDTA (Meals 1, 3, and 5) was significantly decreased in comparison to those supplemented with disodium EDTA (Meals 2 and 4), with the exception of Meal 6, which was fortified with ferric sodium EDTA and had the highest absorption of iron. Addition of disodium EDTA to the meals (Meals 2 and 4) significantly increased iron absorption to a level similar to that of ferric sodium EDTA.

A brief summary of the comparison of the bioavailability of iron from foods fortified with $FeSO_4$ or ferric sodium EDTA is presented in Table 3.1.1-8. In general, iron in the form of ferric sodium EDTA is approximately twice as bioavailable as iron in the form of $FeSO_4$.

Table 3.1.1-8 Comparison of Iron Absorption from Meals of Different Iron Bioavailability Fortified with $FeSO_4$ or Ferric Sodium EDTA; Standardized Iron Absorption (%)[a]				
Components of Meal	**$FeSO_4$**	**FeNaEDTA**	**Ratio FeNaEDTA:$FeSO_4$**	**Reference**
Rice Milk	1.7	4.5	2.6	Viteri *et al*. (1978)
Beans, maize and coffee	2.0	5.3	2.7	Viteri *et al*. (1978)
Bran	2.7	7.8	2.9	MacPhail *et al*. (1981)
Beans, plantain, rice, maize, and soy[b]	3.1	7.0	2.3	Layrisse and Martínez-Torres (1977)
Maize Meal	4.0	8.2	2.1	MacPhail *et al*. (1981)
Beans, plantain, rice, maize, soy, and meat[b]	4.2	7.4	1.4	Layrisse and Martínez-Torres (1977)
Beans, plantain, rice, maize, soy, and meat[b]	4.3	9.6	2.2	Layrisse and Martínez-Torres (1977)
Wheat	6.2	14.6	2.3	Martínez-Torres *et al*. (1979)
Milk	10.2	16.8	1.6	Layrisse and Martínez-Torres (1977)
Sweet Manioc	14.1	16.6	1.2	Martínez-Torres *et al*. (1979)
Sugar Cane Syrup[b]	33.1	10.8	0.3	Martínez-Torres *et al*. (1979)

Adapted from INACG (1993)
[a] Geometric means standardized to a reference absorption of 40%.
[b] Comparison between $FeSO_4$ and ferric sodium EDTA not in the same individuals.

The bioavailability of iron from meals based on corn tortillas and black bean paste fortified with ferrous fumarate, $FeSO_4$, or ferric sodium EDTA was measured in a crossover study of 33 healthy Guatemalan girls (aged 12 or 13 years) *via* a stable-isotope technique based on erythrocyte incorporation (Davidsson *et al*., 2002). The test meals, which consisted of corn tortillas served with refried black bean paste (50 g) and deionised water (250 g), contained 58.3 mg of calcium, 427 mg of phytic acid/serving, 2.2 mg of native iron (unlabelled Fe), and 2 mg of added ^{57}Fe as ferrous fumarate, $FeSO_4$, or ferric sodium EDTA. Venous blood samples were obtained before consumption of the first labelled test meal (Day 1), as well as 14 days after (*i.e.*, on Days 16 and 31 of the study), for analysis of iron status indices (haemoglobin and plasma ferritin) and incorporation of ^{57}Fe into erythrocytes. The subjects were randomly assigned to Studies 1, 2, or 3, and within each study, 5 or 6 girls received either test Meals A or B. Each test meal was given to the subjects twice daily (breakfast and lunch) after an overnight fast for 2 consecutive days. The alternate test meals were subsequently administered 14 days later according to the same study protocol. In Studies 1 and 2, Meal A was fortified with ferrous fumarate, while Meal B contained ferrous fumarate and disodium EDTA at a 1:1 molar ratio relative to total iron (fortification iron plus native iron in the corn masa flour). In Study 3, Meal A was fortified with $FeSO_4$, while Meal B was fortified with $^{57}FeCl_3$ mixed with disodium EDTA at a molar ratio of 1:1 relative to fortification iron, resulting in ferric sodium EDTA. Baseline haemoglobin concentrations of the subjects ranged from 124 to 155 g/L, and baseline geometric mean plasma ferritin concentration was 22 µg/L (5 to 48 µg/L). On Day 16, geometric mean plasma ferritin concentration decreased

to 19 µg/L (5 to 42 µg/L). According to the authors, no improvement in iron bioavailability from ferrous fumarate was observed with the addition of disodium EDTA. In Studies 1 and 2, respectively, geometric mean iron bioavailabilities were 5.5 and 6.2% with no added disodium EDTA, and 6.7 and 5.8% with added disodium EDTA. However, the bioavailability of iron from the test meal was significantly enhanced when $FeSO_4$ was replaced with ferric sodium EDTA. In Study 3, geometric mean iron bioavailability from Meal B, which was fortified with ferric sodium EDTA (9.0%), was significantly increased compared to that from Meal A, which was fortified with $FeSO_4$ (5.5%). The authors concluded that ferric sodium EDTA is effective for use in the fortification of staple foods with high phytic acid content, such as corn masa flour.

Fidler *et al*. (2003) evaluated the absorption of iron from fish sauce or soy sauce fortified with ferric sodium EDTA against a reference fortificant ($FeSO_4$), compared iron absorption from ferric sodium EDTA-fortified fish sauce and soy sauce, and investigated the influence of fish sauce and soy sauce on iron absorption. Five (5) separate iron absorption studies were conducted in healthy adult females (aged 19 to 29 years) in a crossover study design. Ten (10) women were randomly allocated to each of the 5 studies, with each woman acting as her own control. Iron absorption was measured on the basis of erythrocyte incorporation of ^{57}Fe or ^{58}Fe 14 days following consumption of labelled meals (rice or rice and vegetables) with added fish sauce or soy sauce (10 g), which was fortified with 5 mg of iron as ferric sodium EDTA or $FeSO_4$. The total iron content of each test meal ranged from 5.1 to 5.9 mg. Table 3.1.1-9 summarises the iron, calcium, phytic acid, and ascorbic acid contents of the different test meals administered in each of the studies. All test meals were administered, following an overnight fast, on 2 consecutive days. Venous blood samples were obtained on Day 1 and 16 of the study (*i.e.*, 14 days after consumption of the second test meal) for determination of iron status indices (haemoglobin, ferritin, and circulating transferrin receptor). According to the authors, 2 women had iron-deficiency anaemia, while a total of 16 women were noted to be iron-deficient. It was reported that iron absorption from ferric sodium EDTA-fortified fish sauce (3.3%) and soy sauce (6.1%) was not significantly different from that obtained from $FeSO_4$-fortified fish sauce (3.1%) and soy sauce (5.6%). There also was no significant difference in iron absorption between fish sauce (6.7%) and soy sauce (7.9%) fortified with ferric sodium EDTA. While the addition of soy sauce to rice-based meals resulted in a reduction in iron absorption (from 8.5 to 6.0%), the addition of fish sauce produced no significant effects on iron absorption. The absorption of iron from the different rice-based meals administered in the studies is presented in Table 3.1.1-10. According to the authors, the relatively high iron absorption from rice meals containing ferric sodium EDTA-fortified fish sauce or ferric sodium EDTA-fortified soy sauce indicates that these sauces are potentially useful as iron-fortification vehicles.

Table 3.1.1-9 Iron, Calcium, Phytic Acid, and Ascorbic Acid Contents of Test Meals[1]

Study No.	Meal	Source of Fortification Fe	Fortification Fe (mg)	Native Fe (mg)	Calcium (mg)	Phytic Acid[2] (mg)	Ascorbic Acid (mg)
1	Rice, vegetable purée, and fish sauce	$Na^{58}FeEDTA$ or $^{57}FeSO_4$	5	0.4	16.2	25	0.4
2	Rice, vegetable purée, and soy sauce	$Na^{58}FeEDTA$ or $^{57}FeSO_4$	5	0.9	16.0	27	0.4
3	Rice and fish sauce	$^{58}FeSO_4$ or $^{57}FeSO_4$	5	0.2	5.8	25	ND
	Rice		5	0.1	2.0	25	ND
4	Rice and soy sauce	$^{58}FeSO_4$ or $^{57}FeSO_4$	5	0.7	5.6	27	ND
	Rice		5	0.1	2.0	25	ND
5	Rice and fish sauce	$Na^{58}FeEDTA$ or $Na^{57}FeEDTA$	5	0.2	5.8	25	ND
	Rice and soy sauce		5	0.7	5.6	27	ND

Adapted from Fidler *et al*. (2003)

[1] ND – not determined and assumed to be negligible.

[2] The molar ratio of phytic acid to iron was approximately 0.4:1 in all test meals. While soy sauce provided nearly 2 mg phytic acid/meal, nearly all of the phytic acid came from the rice (25 mg/meal). The phytic acid content of the vegetable purée was not measured and assumed to be negligible.

Table 3.1.1-10 Iron Absorption from Rice-Based Meals Served with Iron-Fortified Fish Sauce or Soy Sauce, or Without Any Added Condiment by Healthy Adult Women[1]

Study No.	Meal	Iron Status		Iron Absorption (%)					P^2
		Ferritin (µg/L)	Transferrin Receptor (mg/L)	$FeSO_4$-Fortified Fish Sauce	FeNaEDTA-Fortified Fish Sauce	$FeSO_4$-Fortified Soy Sauce	FeNaEDTA-Fortified Soy Sauce	$FeSO_4$-Fortified (No Sauce)	
1	Rice and vegetable purée	23 (10, 50)	7.6 (6.2, 9.4)	3.1 (1.2, 7.9)	3.3 (1.6, 6.8)	--	--	--	0.66
2	Rice and vegetable purée	14 (9, 23)	8.5 (6.7, 10.8)	--	--	5.6 (3.3, 9.5)	6.1 (3.2, 11.8)	--	0.46
3	Rice	16 (7, 34)	7.5 (1.2, 9.2)	9.5 (4.3, 21.0)	--	--	--	11.6 (5.6, 23.8)	0.14
4	Rice	16 (9, 29)	6.1 (5.4, 6.9)	--	--	6.0 (2.3, 15.8)	--	8.5 (3.8, 19.1)	<0.02
5	Rice	17 (8, 36)	8.5 (6.0, 12.1)	--	6.7 (3.7, 12.1)	--	7.9 (4.7, 13.5)	--	0.08

Fidler *et al*. (2003)
[1] Geometric mean (-1 SD, +1SD); n = 10 women per study.
[2] The statistical analysis was conducted within each study, by paired *t* test.

In a crossover study involving 11 healthy infants (5 boys and 6 girls, aged 18 to 27 weeks), the bioavailability of iron from a wheat- and soy-based infant cereal fortified with ferric sodium EDTA was compared with that from a cereal fortified with $FeSO_4$ plus ascorbic acid (Davidsson *et al*., 2005). Two to 3 weeks before the start of the study, infants were fed 1 or 2 servings/day of a commercial iron-fortified cereal similar to the test meals to ensure acceptance of the cereal product. Each test meal was fed to the infants after an overnight fast, or at least 3 hours after intake of an infant formula on Day 1 of each study. Each study consisted of the ingestion of 2 isotopically labelled test meals (Meals 1 and 2) followed by the collection of faecal material for 72 hours. Capillary blood samples were drawn before and 2 weeks after (*i.e.*, Day 15 of the study) ingestion of Meal 1 for analysis of haemoglobin and plasma ferritin, and incorporation of ^{58}Fe into red blood cells. The second blood sample of Study 1 was used as the baseline sample for Study 2. A final blood sample was obtained 2 weeks after intake of the second labelled test meal (*i.e.*, Day 30 of the study). Meal 1 contained stable-isotope labels of iron ($^{58}FeSO_4$ or $^{58}FeCl_3$), zinc ($^{70}ZnCl_2$), and calcium ($^{44}CaCl_2$), while Meal 2 contained stable-isotope labels of copper ($^{65}CuCl_2$) and magnesium ($^{25}MgCl_2$). The total content of added iron, zinc, and calcium was equilibrated in Meal 2 by the addition of minerals with normal isotopic composition. Iron was added to the test meals as 2.0 mg of $^{58}FeSO_4$, or 2.0 mg of $^{58}FeCl_3$ mixed with disodium EDTA, at an iron to EDTA molar ratio of 1:1. Test meals labelled with $^{58}FeSO_4$ also contained added L-ascorbic acid at a molar ratio of iron to ascorbic acid of 1:1.6. The test meals contained 2.0 ± 0.02 mg iron per 100 g of cereal product, before the addition of the stable-isotope labels. Two (2) of the infants were anaemic, while 1 infant had a low plasma ferritin concentration. Iron bioavailability from a high-phytate, cereal-based complementary food was not significantly different between the 2 iron fortificants (ferric sodium EDTA or $FeSO_4$ plus ascorbic acid). Geometric mean erythrocyte incorporation of iron was 3.7 and 4.9% for cereals fortified with ferric sodium EDTA and $FeSO_4$ plus ascorbic acid, respectively. According to the authors, both iron compounds were equally efficient in providing bioavailable iron from an inhibitory meal since $FeSO_4$ was evaluated in the presence of ascorbic acid. In an earlier study by the same authors, it was reported that the addition of ascorbic acid or disodium EDTA are equally efficient in enhancing iron bioavailability from $FeSO_4$ in a Peruvian school breakfast meal (Davidsson *et al*., 2001).

The absorption of iron from porridges prepared from a genetically modified strain (*lpa-1-1* mutant) of low-phytate maize (LPM) and unmodified wild-type maize (WTM) fortified with either ferric sodium EDTA or $FeSO_4$ was assessed (Mendoza *et al*., 2001). Fourteen (14) healthy, non-pregnant, adult women (aged 19 to 42 years) were provided with porridges fortified with 1 mg iron/serving as $FeSO_4$ or ferric sodium EDTA, and iron absorption was measured as the amount of radiolabelled iron incorporated into red blood cells 12 days after consumption of the test diets. During the study, each subject consumed 2 types of porridges (LPM or WTM fortified with ferric sodium EDTA or $FeSO_4$) for 2 consecutive days each. The total iron content of the porridges was 4.4 mg. The composition of the study diets is presented in Table 3.1.1-11. Blood samples were obtained before and 10 days after (*i.e.*, Day 12 of the study) consumption of the test diets for measurement of iron incorporated into erythrocytes. The second set of test diets was administered to the subjects 2 days after the

last blood sample was obtained (*i.e.*, Day 14 of the study), and a final blood sample was drawn 10 days after (*i.e.*, Day 24 of the study). Four (4) of the subjects had serum ferritin concentrations <12 µg/L, indicating depleted iron stores during baseline, which was not significantly altered during the course of the study. It was reported that the presence of phytate had no significant effect on iron absorption from porridges fortified with either ferric sodium EDTA or $FeSO_4$. Fractional absorption of iron from ferric sodium EDTA-fortified WTM porridge (5.73%) was significantly higher compared to that from $FeSO_4$-fortified WTM porridge (1.69%). In LPM porridges, fractional absorption of iron also was significantly higher in those fortified with ferric sodium EDTA (5.40%) compared to those fortified with $FeSO_4$ (1.91%). The authors concluded that iron absorption was more efficient when fortified with ferric sodium EDTA rather than $FeSO_4$, regardless of the type of maize.

Table 3.1.1-11 Composition of the Test Diets Used in Mendoza *et al*. (2001) Study[1]

Ingredient	Wild-Type Maize (WTM) Porridge		Low-Phytate Maize (LPM) Porridge	
	With FeNaEDTA	With $FeSO_4$	With FeNaEDTA	With $FeSO_4$
Dough (g dry wt.)	76.2	76.2	90.3	90.3
Water (mL)	240	240	240	240
Sugar (g)	22.8	22.8	22.8	22.8
Cinnamon (g)	2.9	2.9	2.9	2.9
Margarine (g)	13.0	13.0	13.0	13.0
Iron (mg)				
Dough	3.4	3.4	3.4	3.4
FeNaEDTA	1.0	--	1.0	--
$FeSO_4$	--	1.0	--	1.0
Total	4.4	4.4	4.4	4.4
Phytate:Iron	16	16	6.8	6.8
Radioiron				
^{55}Fe as $FeCl_3$ (µCi)	2.0	--	2.0	--
^{55}Fe as $FeCl_3$ (kBq)	74	--	74	--
^{59}Fe as $FeCl_3$ (µCi)	--	1.5	--	1.5
^{59}Fe as $FeCl_3$ (kBq)	--	56	--	56

Mendoza *et al*. (2001)
[1] Amounts per serving

In a later study, Mendoza *et al*. (2004) evaluated the effect of a novel fortificant mixture consisting of ferric sodium EDTA, zinc methionine, ascorbic acid, and citric acid on iron and zinc absorption from a dry food supplement designed for pre-school children. Thirteen (13) healthy, non-pregnant, adult women (aged 20 to 31 years) were provided with 1 of 4 food products at 7-day intervals. These food products consisted of a standard or novel food product with either low (192 mg) or high (200 mg) calcium added as $Ca_3(PO_4)_2$. Standard food products contained a fortification mixture of 10 mg of iron as $FeSO_4$, 10 mg of zinc as $ZnSO_4$, and 50 mg of ascorbic acid per 90 g of food, while novel food products contained 10 mg of iron as ferric sodium EDTA, 10 mg of zinc as zinc methionine, 100 mg ascorbic

acid, and 1 g of citric acid. Table 3.1.1-12 presents the composition of the experimental diets. Fasting blood samples were collected on Days 1 and 15 of the study to determine iron incorporation into the red blood cells. Absorption of iron was determined immediately after each test food product was consumed using a whole-body counter. The counts were repeated 7 days later to determine absorption of the previously consumed dose. These measurements also served as the baseline values for the next food product. The same procedure was followed for the remaining test food products, which were fed on Days 15 and 22 of the study. The last blood draw and whole-body counting took place on Day 29 of the study. Two of the subjects were reportedly iron-deficient during the study, with ferritin concentrations of <12 µg/L. Haemoglobin and haematocrit values of subjects did not differ throughout the study period, with mean values of 133 ± 8 g/L and 39.8 ± 2.2%, respectively. The absorption of iron from the novel food product containing ferric sodium EDTA was reported to be 1.7 times higher than that from the standard food product containing $FeSO_4$. Dietary calcium had no significant effect on iron absorption from these products. A significant positive correlation was noted between iron absorption and iron incorporated into red blood cells; however, a significant negative correlation was observed between average iron absorption and serum ferritin values.

Table 3.1.1-12 Composition of the Experimental Diets Used in Mendoza *et al*. (2004) Study

Component	Standard Food Product		Novel Food Product	
	Low Calcium	High Calcium	Low Calcium	High Calcium
Food supplement (g)[1]	90	90	90	90
Iron (mg)				
Food supplement	4.7	4.7	4.7	4.7
$FeSO_4$	10	10	--	--
FeNaEDTA	--	--	10	10
Total	14.7	14.7	14.7	14.7
Zinc (mg)				
Food supplement	1.4	1.4	1.4	1.4
$ZnSO_4$	10	10	--	--
FeNaEDTA	--	--	10	10
Total	11.4	11.4	11.4	11.4
Calcium (mg)				
Food supplement	192	192	192	192
$Ca_3(PO_4)_2$	--	200	--	200
Total	192	392	192	392
Ascorbic acid (mg)	50	50	100	100
Citric acid (g)	--	--	1	1

Table 3.1.1-12 Composition of the Experimental Diets Used in Mendoza *et al*. (2004) Study				
Component	**Standard Food Product**		**Novel Food Product**	
	Low Calcium	**High Calcium**	**Low Calcium**	**High Calcium**
PA[2] (mg)				
Inositol triphosphate	--	--	--	--
Inositol tetraphosphate	6	6	6	6
Inositol pentaphosphate	45	45	45	45
Inositol hexaphosphate	254	254	254	254
Total	306	306	306	306
PA:Zn molar ratio	2.7	2.7	2.7	2.7
Ca:PA molar ratio	10.3	21.1	10.3	21.1
PA:Fe molar ratio	1.8	1.8	1.8	1.8
([Ca] x [PA]):[Zn] molar ratio	0.1	0.3	0.1	0.3

Mendoza *et al*. (2004)
[1] Without addition of iron, zinc, calcium, ascorbic acid, or citric acid
[2] PA – phytic acid

Andang'o and colleagues have recently evaluated the efficacy of iron-fortified whole maize flour on iron status of schoolchildren in Kenya in a randomised controlled trial (Andang'o *et al.,* 2007). They assessed the effect, on children's iron status, of consumption of whole maize flour fortified with iron as ferric sodium EDTA or electrolytic iron. Five hundred sixteen children, aged 3 to 8 years, from four schools in Marafa, Kenya, were randomly assigned to 4 groups. All were given the same amount of porridge 5 times a week. The porridge for 1 group was made from unfortified whole maize flour; for the other 3 groups it was fortified with either high-dose ferric sodium EDTA (56 mg/kg), low-dose ferric sodium EDTA (28 mg/kg), or electrolytic iron (56 mg/kg). Concentrations of haemoglobin, plasma ferritin, and transferrin receptor were analysed in samples taken at baseline and at the end of the 5-month intervention. The primary outcome was iron-deficiency anaemia. Data was analysed on an intention-to-treat basis. This trial was registered with ClinicalTrials.gov, number NCT00386074. The results showed that the prevalence of iron-deficiency anaemia in children given unfortified flour was 10%. Compared with placebo, the prevalence of iron-deficiency anaemia in children given flour fortified with high-dose ferric sodium EDTA, low-dose ferric sodium EDTA, and electrolytic iron changed by -89% (95% CI -97% to -49%), -48% (-77% to 20%), and 59% (-18% to 209%), respectively. Consumption of high-dose ferric sodium EDTA improved all measured iron-status indicators. Low-dose ferric sodium EDTA decreased the prevalence of iron deficiency but did not noticeably change the prevalence of anaemia. Electrolytic iron did not improve any of these iron-status indicators. Children who were iron-deficient at baseline benefited more from high-dose and low-dose ferric sodium EDTA than those with sufficient iron at baseline. The authors concluded that the consumption of whole maize flour fortified with ferric sodium EDTA caused modest, dose-dependent improvements in children's iron status. Fortification with electrolytic iron did not improve their iron status. Therefore, in high-phytate flours, ferric sodium EDTA is more suitable than electrolytic iron for supplementation of iron in the diet.

Table 3.1.1-13 Effect of Consumption of Iron Flour on Iron Status, Compared with Placebo				
	High-dose NaFeEDTA	**Low-dose NaFeEDTA**	**Electrolytic Iron**	**Placebo**
n	121	139	127	128
Haemoglobin concentration (g/L)*	117.2 (8.5)	114.7 (8.8)	112.2 (9.9)	115.7 (9.7)
Crude effect	1.6 (-0.7 to 3.8)	-0.7 (-2.9) to 1.5	-3.5 (-5.7 to -1.2)	Reference
Adjusted effect[1]	4.0 (2.3 to 5.6)	1.3 (-0.3 to 2.8)	-1.3 (-2.9 to 0-3)	Reference
Plasma ferritin concentration (µg/L)[2]	35.0 (24.5 to 47.0)	28.0 (20.0 to 39.0)	23.0 (13.0 to 32-0)	23.0 (16.0 to 36.0)
Crude effect[3]	54% (33% to 78%)	23% (7% to 41%)	-3% (16% to 12%)	Reference
Adjusted effect[1,3]	67% (49% to 89%)	36% (-21% to 53%)	6% (-5% to 20%)	Reference
Plasma soluble transferring receptor concentration (mg/L)[2]	2.3 (1.9 to 2.7)	2.4 (2.0 to 2.8)	2.6 (2.1 to 3.2)	2.5 (2.1 to 3.1)
Crude effect[3]	-11% (-17% to -5%)	-8% (-14% to -2%)	4% (-3% to 11%)	Reference
Adjusted effect[1]	-15% (-19% to -11%)	-12% (-16% to -8%)	0% (-4% to 5%)	Reference
Anaemia[4]	38 (31.4%)	68 (41.7%)	65 (51.2%)	48 (37.5%)
Crude effect	-16 (-45 to 28)	11 (24 to 63)	36 (-6 to 98)	Reference
Adjusted effect[1]	-36 (-58 to -1)	-2 (-33 to 44)	12 (-23 to 63)	Reference
Iron deficiency[6]	3 (2.5%)	14 (10.0%)	33 (26.0%)	27 (21.1%)
Crude effect[5]	-88% (-96 to -61)	-52% (-75 to -9)	23% (-26 to 105)	Reference
Adjusted effect[1,5]	-91% (-97 to -49)	-70% (-85 to -40)	1% (-40 to 69)	Reference
Iron-deficiency anaemia[7]	2 (1.7%)	12 (8.6%)	27 (21.3%)	13 (10.2%)
Crude effect[5]	-84% (-96 to -28)	-15% (-61 to 86)	109% (8 to 306)	Reference
Adjusted effect[5]	-89% (-97 to -49)	-48% (-77 to 20)	59% (-18 to 209)	Reference

Values are mean [95% CI] or number (%) unless indicated otherwise. Treatment effects are measured as group differences (continuous outcomes) relative to placebo. All analyses are by intention to treat. *Mean (SD). [1] Effect of intervention adjusted for baseline factors: haemoglobin concentration, plasma concentrations of ferritin, soluble transferrin receptor and C-reactive protein, and malaria antigenaemia. [2] Median (IQR). [3] Values indicate difference between groups, expressed as a percentage relative to the placebo group, obtained by exponentiation of effect estimates from log-transformed data. [4] Anaemia defined as haemoglobin concentration <110 g/L for children aged >5 years and >115 g/L for those >5 years. [5] Values indicate percentage difference in prevalence as compared with placebo [95% CI], obtained by conversion of prevalence ratios from Cox regression with constant time at risk. [6] Iron deficiency defined as plasma ferritin concentration <12 µg/L for those=5 years. [7] Iron deficiency anaemia defined as concurrent anaemia and iron deficiency.

3.1.2 Animal Studies

Yeung *et al.* (2004) conducted an iron absorption study to compare the down-regulation of iron absorption from $FeSO_4$ and ferric sodium EDTA in rats. Two groups of 9 male Sprague-Dawley rats were fed basal diets for a period of 29 days, while 2 additional groups were fed diets containing 30,000 mg elemental iron/kg diet to induce iron loading over the same period. On Day 30, 1 group of rats fed the basal diet and 1 group fed the high-iron diet were administered diets providing 35 mg iron/kg diet as radiolabelled $FeSO_4$ or ferric sodium EDTA. The rats were fed their respective unlabeled diets (*i.e.,* unlabeled $FeSO_4$ or ferric sodium EDTA) for another 10 days. There was no significant difference between groups in blood haemoglobin concentration. Rats fed the high-iron diet had significantly higher tissue non-haem iron concentration compared to those fed the basal diet. There was no significant difference between $FeSO_4$-fed and ferric sodium EDTA-fed rats with respect to tissue non-haem iron concentrations. Among rats fed the basal diets, iron retention and absorption was significantly greater in rats fed $FeSO_4$ 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. The authors concluded that absorption of iron is downregulated in iron-loaded rats, and that ferric sodium EDTA is "no more likely than $FeSO_4$ to exacerbate iron overload in subjects with adequate body iron stores."

3.1.3 *In Vitro* Studies

In an *in vitro* digestion/Caco-2 cell culture model, the availabilities and dialyzabilities of various iron fortificants (ferric sodium EDTA, ferrous bisglycinate, $FeSO_4$, electrolytic iron, encapsulated ferrous fumarate, or ferrous fumarate) were compared in bread and milk (Yeung *et al.*, 2002). Each loaf of white bread was prepared from flour fortified with 44 mg iron/kg, and allowed to equilibrate to room temperature before being freeze-dried and crushed. The iron content of crushed, freeze-dried, fortified breads was adjusted by mixing with crushed, freeze-dried, unfortified breads, resulting in an iron content of approximately 47 µg/g. In addition, a sugar/iron fortificant mixture was prepared by mixing powdered sugar with iron fortificant at a level sufficient to provide approximately 20.94 mg of iron. On the day of the experiment, pasteurised milk (2% fat) was fortified with the sugar/iron fortificant mixture, providing an iron concentration of 12 mg/L of milk. Subsequently, aliquots of 0.89 g of freeze-dried bread or 20 mg of sugar/iron fortificant mixture plus 3.5 mL of milk (2% fat) were placed in 50 mL capped tubes containing pepsin/pancreatin-bile extract digest providing final iron concentrations of 50 µmol/L. The intracellular ferritin concentration (ng ferritin/mg cell protein) served as an index of iron uptake by Caco-2 cells, and therefore represented the availability of iron from the food samples. The amount of iron dialyzed also was estimated by determining the concentration of iron in the bottom chamber media plates. Iron dialyzability and availability were significantly higher in breads fortified with ferrous bisglycinate, ferrous fumarate, or $FeSO_4$ compared to unfortified or electrolytic iron-fortified bread. While ferric sodium EDTA-fortified bread provided the highest amount of dialyzable iron, it had a significantly lower iron availability compared to breads fortified with ferrous bisglycinate, ferrous fumarate, or $FeSO_4$. Similarly, the availability and dialyzability of iron

from fortified milk were significantly higher compared to those from unfortified milk; however, the availability of iron from fortified milk was not significantly different between iron fortificants. The amount of dialyzed 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 was used.

The iron availabilities of ferric sodium EDTA, ferrous bisglycinate, $FePO_4$, $FeSO_4$, $FeCO_3$, encapsulated ferrous fumarate, ferrous lactate, carbonyl iron, electrolytic iron, Biofer ($FeSO_4$), SQM (polysaccharide-complexed $FeSO_4$), SunActive (ferric pyrophosphate), and reduced iron (control) in a wheat-based cereal was investigated in an *in vitro* digestion/ Caco-2 model (Wortley *et al*., 2002). All of the iron-fortified cereals were reported to exhibit an increased iron availability compared to unfortified cereal. In particular, iron availabilities were higher than control in this order: ferric sodium EDTA (291%) > ferrous bisglycinate (125%) > SunActive (78%) > electrolytic iron (52%) > encapsulated ferrous fumarate (30 to 35%).

The amount of dialyzable iron after simulated gastrointestinal digestion of flour baked into chapatis and subsequent intestinal absorption of the released iron using Caco-2 cell layers was investigated (Kloots *et al*., 2004). According to the authors, iron dialyzability from unfortified wheat flour was extremely low (0.6 ± 0.3 mg dialyzable iron/kg of flour, or 2.1 ± 0.8% of total iron), and additions of 50 mg/kg of iron to the flour in the form of $FeSO_4$, Ferrochel ferrous amino acid chelate, ferric amino acid chelate taste-free, Lipofer (a complex of ferric pyrophosphate, starch, and lecithin), ferrous lactate, ferrous fumarate, ferric pyrophosphate, carbonyl iron, or electrolytic iron produced no significant increases in the amount of dialyzable iron after simulated gastrointestinal digestion. In contrast, fortification of wheat flour with iron from SunActive or Ferrazone® ferric sodium EDTA resulted in a significant increase in the amount of dialyzable iron relative to $FeSO_4$ (increases of 4- and 7-fold, respectively). However, when compared to $FeSO_4$-fortified digested chapatis, only those fortified with ferric sodium EDTA produced a significantly higher absorption of iron in Caco-2 cells.

Results of another study that used an *in vitro* enzymatic digestion method, simulating conditions in the small intestine, showed a significantly higher percentage of dialyzable iron from ferric sodium EDTA- or $FeSO_4$-fortified rice compared to that from rice fortified with ferrous fumarate or ferrous bisglycinate (Trinidad *et al.,* 2002). The iron fortificant was dispersed in a binder solution consisting of a mixture of carboxy ethylcellulose, carboxy methylcellulose, and organic solvents (isopropyl alcohol, ethanol, and absolute alcohol), and subsequently poured onto rice grains tumbling in a rotary mixer. Subsequently, the coated iron-fortified rice samples were air-dried for 24 hours and packed in polyethylene bags. In this study, the amount of iron-fortified rice used provided approximately 3 mg iron/100 g of rice. The percentage of iron dialyzability in the fortified rice samples (cooked) was reported to be in this order: ferric sodium EDTA (15.7) > $FeSO_4$ (13.2) > ferrous fumarate (6.4) > unfortified rice (4.4) > ferrous bisglycinate (3.3). No significant differences were observed in the percentages of dialyzable iron between ferric sodium EDTA- and $FeSO_4$-fortified rice.

3.1.4 Bioavailability Data on Analogous Substances

See Sections 3.1.1, 3.1.2, and 3.1.3.

3.2 Metabolic Fate and Biological Distribution

3.2.1 General Metabolism of Iron from Organic and Inorganic Sources

Absorption of orally administered iron may occur *via* 1 of 2 pathways, depending on whether it is of the organic (haem) or inorganic (non-haem) form. Dietary haem iron, which primarily comes from haemoglobin and myoglobin in meat, is absorbed into the intestinal cells as the intact porphyrin complex (INACG, 1993; IOM, 2001). Iron is subsequently released from this complex by the haem oxygenase enzyme and transferred into the blood stream for transport, together with other iron taken up by the cells (INACG, 1993). Haem iron is highly bioavailable (20 to 25% absorption) and is relatively unaffected by dietary factors (Viteri *et al*., 1978; INACG, 1993; IOM, 2001). Non-haem iron, which is derived from various food sources (*e.g.*, vegetable foods, dairy products, non-haem meat iron and dietary iron fortificants), is solubilised and transferred into a common non-haem iron pool located in the lumen of the upper gastrointestinal tract (INACG, 1993; IOM, 2001). In contrast to haem iron, the amount of non-haem iron absorbed from this pool is greatly affected by the presence of ligands in undigested or partially digested foods, which either enhance (*e.g.,* ascorbic acid), or inhibit (*e.g.*, polyphenols, phytate) absorption (INACG, 1993; IOM, 2001). Additionally, some insoluble form of iron may be ingested, which does not enter the common non-haem iron pool, and thus is not absorbed (INACG, 1993). The iron from ferric sodium EDTA undergoes the absorptive pathway for the inorganic, non-haem form of iron.

Following absorption, iron is distributed throughout the body reversibly bound to transferrin for utilization in proteins, including storage proteins (*i.e.*, ferritin and haemosiderin), transport proteins (*i.e.*, transferrin or lactoferrin), haem-containing proteins (*i.e.*, haemoglobin, myoglobin, cytochromes), and enzymes (*i.e.*, iron-containing or activated non-haem enzymes, iron-sulphur enzymes or flavoproteins) (IOM, 2001). Iron utilized in storage proteins make up about 20 to 30% of the total iron in the body, with primary iron storage sites identified in the cells of the liver, spleen and bone marrow (Appel *et al*., 2001; IOM, 2001). About 60 to 70% of total body iron is present in the haemoglobin of circulating erythrocytes, while 10% has been identified in myoglobin, cytochromes, and other iron-containing enzymes (Appel *et al*., 2001; IOM, 2001). The amount of iron in the body is highly conserved, with daily basal iron losses of 0.2 mg in infants, 0.5 mg in children aged 6 to 11 years, 0.6 to 1.0 mg in men, 0.64 mg in non-menstruating women, and 1.2 to 1.3 mg in menstruating women (Green *et al*., 1968; UK EVM, 2003). The majority of iron losses have been reported to be due to faecal excretion (Green *et al*., 1968; IOM, 2001). Iron losses also may be due to losses from the urine, gastrointestinal tract and skin, which contribute approximately 0.08, 0.6 and 0.2 to 0.3 mg of the iron, respectively, that is lost from the body per day (Green *et al*., 1968; IOM, 2001).

Following oral administration of ferric sodium EDTA in pigs and in humans, the iron from ferric sodium EDTA is separated from the ferric EDTA complex in the lumen of the gut, joining the general non-haem iron pool that is finally incorporated into the circulating haemoglobin (Candela *et al*., 1984). Thus, iron from ferric sodium EDTA is subject to the same controls as other forms of iron. In humans, less than 1% of the intact ferric EDTA chelate is absorbed, which, in turn, is rapidly excreted in the urine within 24 hours (Martínez-Torres *et al*., 1979; MacPhail *et al*., 1981; Candela *et al*., 1984). Iron absorption is precisely regulated by down-regulation systems in the body (Hallberg *et al*., 1997; Yeung *et al*., 2004); therefore, iron fortification is not expected to result in iron overload in otherwise normal populations.

3.2.2 Metabolic Fate of Ferric Sodium EDTA

Candela *et al*. (1984) conducted iron absorption studies on 16 male Yorkshire-Hampshire pigs administered 5 mg of iron (approximately 0.13 mg iron/kg body weight) orally through a gelatine capsule containing 36 mg $Na^{55}Fe$-[2-^{14}C]EDTA. For control, pre-intervention blood, urine, and faecal samples from all of the pigs were obtained. Samples of blood, urine, and faeces were collected from 3 of the animals prior to euthanasia, at 1, 2, or 5 hours following $Na^{55}Fe$-[2-^{14}C]EDTA administration. Blood, urine, and faecal samples also were obtained from the remaining 13 animals for a period of up to 48 hours. Within 1, 2, and 5 hours of $Na^{55}Fe$-[2-^{14}C]EDTA ingestion, the majority of ^{55}Fe and ^{14}C were absorbed in the jejunum, while only minor amounts were absorbed in the duodenum. In contrast to ^{55}Fe, it was reported that only a small percentage of ^{14}C could be detected in the plasma at any time. The authors reported that an average of 4.3% of the administered ^{55}Fe dose was incorporated into haemoglobin, while 0.3 and 5.4% of the administered ^{55}Fe and ^{14}C doses, respectively, were excreted in the urine of animals within 48 hours. The highest radioactivity was detected in the faeces of pigs between 18 and 30 hours. Mean 48-hour faecal excretion ranged from 72 to 91% of the administered ^{55}Fe dose, with only 3 to 4% of ^{55}Fe identified as the soluble ^{55}Fe-EDTA complex, indicating that the remaining 96 to 97% was transformed into an insoluble ^{55}Fe compound.

Additional iron absorption studies were conducted in 6 fasting volunteers (1 male and 5 females) by the administration of a drinking solution containing 5 mg of iron as ^{59}Fe(III)EDTA (approximately 0.083 mg iron/kg body weight) (Candela *et al*., 1984). Urine samples were collected from each of the subjects for a period of 48 hours, while blood samples were obtained 15 days following ^{59}Fe(III)EDTA administration. For control, pre-intervention measurements of blood haemoglobin concentration, serum iron concentration and unsaturated iron capacity were obtained from each of the subjects. Anaemia was reported in 1/5 of the subjects, while 3/5 was reported to show transferrin saturation below 15%. Mean iron absorption was 12.0%, as determined from the incorporation of ^{59}Fe into the circulating haemoglobin, with 0.3% of the absorbed ^{59}Fe being eliminated in the urine. The majority of the ^{59}Fe administered was identified in the urine of the subjects within 24 hours; however, this was only equivalent to <1% of the administered dose. The authors concluded that, in humans, iron absorption from ferric sodium EDTA is related to body iron reserves,

but not directly related to urinary iron excretion, such that urinary iron excretion does not increase in parallel with iron absorption.

Similar conclusions were reached when the 24-hour urinary excretion of ^{59}Fe(III)EDTA was studied in 14 healthy subjects administered 100 mL of water sweetened with ^{59}Fe(III)EDTA-fortified sugar (MacPhail *et al*., 1981). All of the ^{59}Fe was excreted in the urine of subjects within the first 24 hours; however, this represented only <1% of the administered dose, which the authors attributed to the absorption of ^{59}Fe in the form of the intact ^{59}Fe(III)EDTA. The authors reported that a significant inverse relationship existed between the percentage excretion and absorption of ^{59}Fe, which they suggested to be due to the absorption of the non-chelated form of iron by the intestinal mucosa.

In oral absorption studies of Fe(III)EDTA conducted in 3 healthy subjects by Martínez-Torres *et al*. (1979), about 16% of the total iron absorbed was excreted in the urine of subjects, and as much as 49% of the administered dose was identified in the red blood cells of iron-deficient subjects. Additionally, *in vitro* experiments using human serum demonstrated that transferrin does not bind iron from ^{59}Fe(III)EDTA. According to the authors, this is due to the separation of ^{59}Fe from EDTA during intestinal absorption, such that only a small proportion of iron enters into the circulation as the Fe-EDTA complex, which is in turn, subsequently excreted in the urine.

The effects of administering iron-fortified foods in iron-repleted and iron-depleted states were examined in 31 males (12 of whom were blood donors), who received iron-fortified meals 4 times/day for a period of 5 days (Hallberg *et al*., 1997). The non-haem and haem iron in these meals were radiolabelled with ^{59}Fe (as $^{59}FeCl_3$) and ^{55}Fe (as ^{55}Fe-labeled rabbit haemoglobin), respectively. Two weeks after the subjects received their last serving of iron-fortified meals, the total ^{59}Fe was measured in a whole-body counter and the absorption ratio of the 2 tracers (^{59}Fe and ^{55}Fe) was determined from the blood samples to obtain the total ^{55}Fe retention of each subject. The reference retention value of ^{59}Fe was obtained by administering 3 mg of $FeSO_4$ + 30 mg of ^{59}Fe-labeled ascorbic acid to fasting subjects twice within a 24-hour period. The retention of absorbed ^{59}Fe from the reference dose was measured 2 weeks later. The absorption of haem iron, non-haem iron and total iron were significantly higher in blood donors than in non-donors; however, serum ferritin levels were significantly lower in blood donors than in non-donors. An inverse relationship was noted between serum ferritin and iron absorption values, such that as serum ferritin levels increased, the absorption of both haem and non-haem iron decreased, with the absorption of non-haem iron decreasing more than that of haem iron. Haem iron absorption was reported to be 40, 80, and 140% more at serum ferritin levels of 15, 20, and 30 µg/L, respectively. Comparison of haem and non-haem iron absorption revealed that there was a steep decrease in haem-iron absorption with decreasing non-haem iron absorption. The authors reported that in iron-depleted states (*i.e.*, serum ferritin level of 10 µg/L), the absorption of haem and non-haem iron is similar; however, in iron-repleted states (*i.e.*, serum ferritin level >70 µg/L), decreases in iron absorption was more marked for non-haem iron, which suggests that haem-iron absorption may be responsible for a greater part of the total iron absorption in iron-repleted subjects. Therefore, the authors suggested that there exists a

very effective control for non-haem and haem iron absorption, ensuring that an accumulation of iron will not be a source for concern in healthy, iron-repleted subjects. In a similar manner, the authors reported that the body regulates iron absorption from fortified foods in the same way as dietary non-haem iron, which has been shown to be especially critical in iron-depleted subjects.

3.3 Interactions with Other Components in the Diet

See Section 3.4.

3.4 Impact on the Intestinal Milieu and on the Absorption of Other Nutrients

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 use of ferric sodium EDTA has been reported to enhance the absorption and retention of copper and zinc from the diet (Hurrell *et al*., 1994). In humans, fortification of foods with ferric sodium EDTA resulted in no effects 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 (Solomons *et al*., 1979; Davidsson *et al*., 1994; Mendoza *et al*., 2004). The apparent absorption of zinc, copper, calcium, or magnesium was reportedly similar between meals fortified with ferric sodium EDTA and $FeSO_4$ plus ascorbic acid (Davidsson *et al*., 2005).

3.4.1 Animal Studies

The effect of dietary ferric sodium EDTA on the metabolism of zinc, copper, and calcium was studied in groups of male weanling Sprague-Dawley rats (6 to 8/group) (Hurrell *et al.*, 1994). Basal diets contained 18.5 mg iron/kg diet, 4.95 g calcium/kg diet, 9.7 mg copper/kg diet, and 6.1 mg zinc/kg diet. A total of 8 test diets were formulated; Diets A to D were supplemented with zinc to provide a total zinc concentration of 30 mg/kg diet (zinc-sufficient diets), while Diets E to H were not supplemented with zinc (zinc-deficient diets). All diets were supplemented with 31.6 mg iron/kg diet to provide a total concentration of 50.1 mg iron/kg diet (approximately 7.20 mg iron/kg body weight/day) as $FeSO_4 \cdot 7H_20$ (Diets A and E), or as ferric sodium EDTA (Diets B, C, D, F, G, and H; providing 200 mg EDTA/kg diet). Diets C, D, G, and H were further supplemented with disodium EDTA?H_2O, providing an additional 300 mg EDTA/kg diet in groups C and G (total of 500 mg EDTA/kg diet, or 71.8 mg EDTA/kg body weight/day), and an additional 800 mg EDTA/kg diet in groups D and H (total of 1,000 mg EDTA/kg diet, or 143.7 mg EDTA/kg body weight/day). All diets were administered for a period of 21 days. Body weight gain and food consumption were recorded every 3 to 4 days, while a 4-day urine and faeces collection was initiated on Day 18 of the study. The animals were killed on Day 21 of the study and the right femur was removed for analysis. The results of the study are summarised in Tables 3.4.1-1 and 3.4.1-2.

The source of iron ($FeSO_4$ in Diet A *vs.* ferric sodium EDTA in Diet B) had no significant effect on food intake, body weight gain, or femur zinc and calcium concentrations in rats fed

zinc-deficient diets; however, femur zinc concentrations were significantly reduced in rats fed higher concentrations of EDTA (Diets C and D). In contrast, neither the source of iron nor the concentration of EDTA in the diet had any significant effect on femur zinc and calcium concentrations in rats fed the zinc-deficient diets (Diets E to H). However, rats fed zinc-deficient diets supplemented with ferric sodium EDTA (Diet F) had significantly higher food intake and body weight gain in comparison to those fed zinc-deficient diets supplemented with $FeSO_4$ (Diet E). Food consumption and body weight gain tended to decrease with consumption of zinc-deficient diets with higher levels of EDTA (Diets G and H); however, food intake and body weight gain in rats fed 1,000 mg EDTA/kg diet (Diet H) was not significantly different from rats fed diets without EDTA (Groups A and E).

Table 3.4.1-1 The Reported Effects of Different Dietary EDTA Levels on Average Food Intake, Weight Gain, Femur Zinc, and Calcium Concentrations in Rats

Group	No. of Rats	Dietary EDTA[#] (mg/kg diet)	Food Intake* (g)	Weight Gain* (g)	Femur Zinc Levels (mg/g)	Femur Calcium Levels (mg/g)
Zinc-sufficient Diet (30 mg zinc/kg diet)						
A	6	0	273[a]	129[a]	180[a,b]	188[a]
B	7	200	272[a]	129[a]	184[a]	181[a]
C	7	500	273[a]	122[a]	166[b]	175[a]
D	8	1,000	259[a]	127[a]	168[b]	188[a]
Zinc-deficient Diet (6.1 mg zinc/kg diet)						
E	8	0	222[a]	89[a]	72[a]	182[a]
F	6	200	248[b]	106[b,c]	81[a]	172[a]
G	7	500	258[b]	113[b]	75[a]	177[a]
H	7	1,000	230[a,b]	98[a,c]	73[a]	190[a]

Hurrell *et al*. (1994)
[a, b, c] Within Groups A-D and within Groups E-H, means with unlike superscript letters were significantly different ($P < 0.05$).
* Average values calculated over a period of 21 days.
[#] In the diets of Groups B and F, total dietary EDTA content was derived from ferric sodium EDTA, which replaced the $FeSO_4$ that was included in the diets of Groups A and E rats; in Groups C, D, G, and H, dietary EDTA was added as Na_2EDTA, in addition to ferric sodium EDTA.

The absorption, urinary excretion, and retention of zinc was reported to be higher in all groups fed EDTA compared to those fed $FeSO_4$, regardless of the level of zinc in the diet; however, statistically significant differences in all parameters were observed only in rats fed zinc-deficient diets containing EDTA (Diets F, G, and H). Urinary excretion of zinc also was significantly increased in rats fed zinc-sufficient diets supplemented with 500 and 1,000 mg EDTA/kg diet (Diets C and D), while zinc retention was significantly increased following consumption of zinc-sufficient diets supplemented with 500 mg EDTA/kg diet (Diet C), in comparison to those fed diets containing $FeSO_4$. With respect to the apparent absorption, urinary excretion or retention of copper and calcium, neither dietary zinc nor EDTA levels had a significant effect. In rats administered zinc-deficient diets, urinary copper excretion was significantly increased only in rats provided with 500 mg EDTA/kg diet (Group G), while urinary calcium excretion was significantly increased in rats fed 500 and 1,000 mg EDTA/kg diet (Groups G and H, respectively).

Table 3.4.1-2 The Reported Effects of Different Dietary EDTA Levels on the Average Values of 4-Day Zinc Balance in Rats

Group	No. of Rats	Dietary EDTA[#] (mg/kg)	Zinc Intake (mg)	Apparent Zinc Absorption*	Urinary Zinc*	Zinc Retention*
Zinc-Sufficient Diet (30 mg zinc/kg diet)						
A	6	0	1.62[a,b]	16.0[a]	0.7[a]	15.3[a,c]
B	7	200	1.52[a]	24.2[a,c]	1.7[a]	22.5[a,b]
C	7	500	1.69[b]	30.4[b,c]	3.4[b]	27.0[b]
D	8	1,000	1.50[a]	20.4[a]	5.9[c]	14.5[c]
Zinc-Deficient Diet (6.1 mg zinc/kg diet)						
E	8	0	0.23[a]	50.2[a]	2.0[a]	48.2[a]
F	6	200	0.29[a]	67.4[b]	4.0[b]	63.4[b]
G	7	500	0.33[a]	79.4[c]	6.7[c]	72.7[c]
H	7	1,000	0.25[a]	78.1[c]	15.6[d]	62.5[b]

Hurrell *et al*. (1994)

[a, b, c, d] Within Groups A-D and within Groups E-H, means with unlike superscript letters were significantly different ($P < 0.05$).

* Average % values calculated as % intake.

[#] In the diets of Groups B and F, total dietary EDTA content was derived from ferric sodium EDTA, which replaced the $FeSO_4$ that was included in the diets of Groups A and E rats; in Groups C, D, G, and H, dietary EDTA was added as Na_2EDTA, in addition to ferric sodium EDTA.

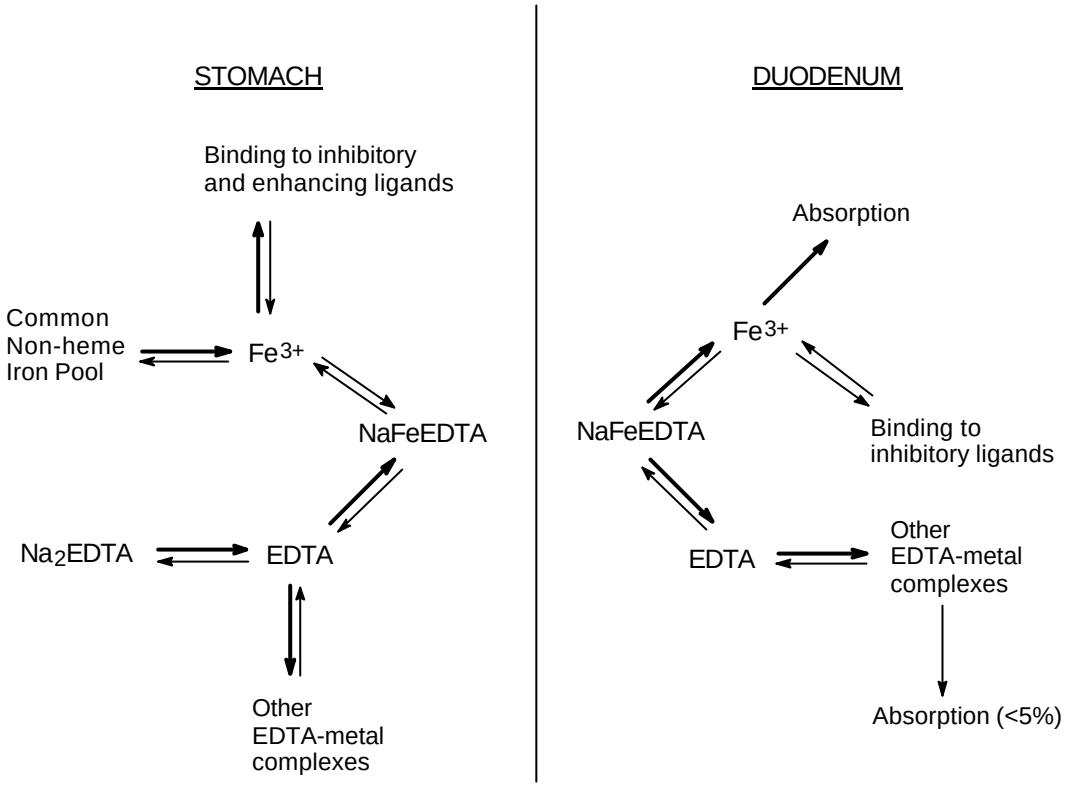
Based on the above results, the authors concluded that the use of ferric sodium EDTA as a food additive, at dietary levels of up to 4.5 mg fortified iron/kg body weight/day and a total of 144 mg EDTA/kg body weight/day, would have beneficial effects on the metabolism of nutritionally-important minerals, such as zinc and copper, while having no effect on the metabolism of calcium in rats. This effect was particularly evident in rats fed a zinc-deficient diet, as significant improvements in copper absorption, zinc absorption and retention, as well as increased weight gain, were observed when ferric sodium EDTA in place of $FeSO_4$ as the source of iron in the diet. The enhanced zinc and iron absorption in rats administered dietary EDTA was attributed to the formation of soluble metal-EDTA chelates, which are highly absorbable and subsequently prevent the reaction of metals with other ligands that could potentially inhibit absorption, such as phytate. Therefore, the formation of highly absorbable zinc-EDTA chelate resulted in an increased urinary excretion of zinc in the rats.

3.4.2 Human Studies

Solomons *et al*. (1979) studied the effects of ferric sodium EDTA on the absorption of zinc from food and water in healthy adult volunteers living in Guatemala. The subjects were administered a meal consistent with the traditional rural Guatemalan diet, with the exception of the black bean gruel, which was mixed with 25 mg of elemental zinc as 110 mg of $ZnSO_4 \cdot 7H_2O$ (approximately 0.42 mg zinc/kg body weight) and coffee that was sweetened with 15 g of sugar fortified with 15 mg ferric sodium EDTA. The subjects also were provided with aqueous solutions containing 25 mg of zinc and 15 mg of disodium EDTA (approximately 0.25 mg disodium EDTA/kg body weight), or ferric sodium EDTA at doses of 15, 40, 115, or 308 mg (approximately 0.25, 0.67, 1.92, and 5.13 mg ferric sodium EDTA/kg

body weight, respectively, which correspond to iron intakes of approximately 0.03, 0.08, 0.24 and 0.65 mg/kg body weight, respectively). For control, the absorption of 25 mg of zinc in 100 mL of water was measured in 5 fasting subjects; while in 2 other subjects, the absorption of 25 mg of zinc in 100 mL of water was measured, with or without 250 mL of coffee sweetened with 15 g of ordinary table sugar. Four (4) hours after the consumption of a standard Guatemalan meal (*i.e.*, not fortified with zinc or iron) significantly decreased plasma zinc levels were observed. Addition of 25 mg of zinc to the meal slightly increased plasma zinc levels, but not above fasting levels. It was reported that in 5 subjects provided with black bean gruel containing 25 mg of zinc and coffee sweetened with 15 g of ferric sodium EDTA-fortified sugar, plasma zinc levels were not significantly different from controls. There were no significant differences in plasma zinc levels following consumption of aqueous solutions containing 15 or 40 mg of ferric sodium EDTA compared to controls; however, a significant decline in plasma zinc levels was reported when aqueous solutions containing 115 or 308 mg of ferric sodium EDTA were consumed. According to the authors, the steep decline in zinc absorption started below the zinc to EDTA molar ratio of 3:1. To determine which of the iron or EDTA moieties of ferric sodium EDTA was responsible for the inhibition of zinc uptake, aqueous solutions containing 39 mg of elemental iron or 240 mg of EDTA (*i.e.*, iron and ferric sodium EDTA doses equivalent to 308 mg of ferric sodium EDTA) were given to 2 different subjects. With the administration of 39 mg iron, plasma zinc levels were not significantly different from controls; while significantly reduced plasma zinc levels were observed with administration of 240 mg of EDTA compared to controls. Therefore, the authors concluded that it was the EDTA moiety that produced inhibitive effects on zinc absorption, at doses of 115 and 308 mg of ferric sodium EDTA. Based on these results, the authors suggested that, unlike the impairment in zinc metabolism observed with consumption of a standard Guatemalan diet, fortification of sugar with ferric sodium EDTA at a level of 1,000 mg/kg would not interfere with zinc nutriture. The proposed mechanism of interaction between zinc and metal ions in the stomach and duodenum is illustrated in Figure 3.4.2-1.

Figure 3.4.2-1 Interaction Between EDTA and Metal Ions in the Lumen of the Stomach and Duodenum



INACG (1993)

The effect of ferric sodium EDTA on zinc and calcium metabolism was examined in a 28-day crossover study (Davidsson *et al*., 1994). A total of 10 healthy women were randomly assigned to 1 of 2 groups, wherein they received diets that included wheat bread fortified with iron as $FeSO_4$ or ferric sodium EDTA for periods of 14 days, with a 4-week washout period between diets. The diets were reported to contain a total iron content of 18 mg/ person/day, with 10 mg of iron provided from the fortification of the wheat bread, as well as 12 mg/person/day of zinc, 800 mg/person/day of calcium and approximately 60 mg/person/ day of ascorbic acid (approximately 0.3 mg iron/kg body weight/day, 0.2 mg zinc/kg body weight/day, 13.3 mg calcium/kg body weight/day and 1 mg ascorbic acid/kg body weight/day, respectively). For control, pre-intervention iron-status indexes, as well as zinc and calcium serum concentrations, were obtained from blood and urinary samples collected from each of the subjects on Day 6 of the study. Stable isotopes of zinc (0.9 mg Zn as $^{70}ZnCl_2$) and calcium (24 mg Ca as $^{44}CaCl_2$) were administered to the subjects on Day 6, and urine was collected from Days 6 to 14. To ensure constant mineral intake throughout the study, unlabelled 0.9 mg Zn and 24 mg Ca were added to the bread on days when the isotopes were not administered. Consumption of ferric sodium EDTA-fortified bread resulted in significantly increased apparent ^{70}Zn absorption and urinary excretion of ^{70}Zn and total zinc, as compared with consumption of $FeSO_4$-fortified bread; however, the authors

suggested that since urinary ^{70}Zn excretion represented <0.1% of the administered dose, the nutritional impact of this result can be considered negligible. There were no significant differences in the retention of ^{70}Zn and total zinc reported between the 2 iron-fortified bread meals, which the authors attributed to the very small amount of zinc excreted in the faeces. Consumption of ferric sodium EDTA-fortified bread also was reported to significantly increase urinary excretion of ^{44}Ca as compared with consumption of $FeSO_4$-fortified bread; however, there was no significant change in the subjects' urinary excretion of total calcium throughout the duration of the study. Additionally, no significant differences in ^{44}Ca and total calcium absorption and retention were observed between the 2 iron-fortified bread meals. The authors concluded that the addition of ferric sodium EDTA to foods would not have detrimental effects on the metabolism of zinc and calcium in humans. In addition, they concluded that the addition of ferric sodium EDTA to foods with a low bioavailability might result in increased zinc absorption and iron bioavailability, which will be particularly important in developing countries where low-bioavailable foods form the basis of the staple diet.

A total of 53 Sri Lankan children aged 7 to 10 years were randomly divided into 4 groups (6 to 8 per sex per group) and fed diets containing rice flour fortified with 60 mg iron (as $FeSO_4$)/kg flour and folate (2 mg/kg flour) (Hettiarachchi *et al.*, 2004). The rice flour was fortified as follows: $FeSO_4$ + folate (Group 1), $FeSO_4$ + folate + disodium EDTA (385.08 mg/kg flour) (Group 2), $FeSO_4$ + folate + 60 mg zinc (as ZnO) (Group 3), and $FeSO_4$ + folate + disodium EDTA + ZnO (Group 4). Subjects consumed the fortified diets for a period of 14 days prior to an absorption trial, in which subjects consumed their respective tests diets, which were prepared with radiolabelled iron and/or zinc. Baseline measurements indicated that 38% of subjects were anaemic, 8% had low serum ferritin, 36% were deficient in folate, and 15% were deficient in zinc. The disodium EDTA-supplemented groups (2 and 4) were reported to have significant increases from baseline in serum haemoglobin, serum ferritin, and serum zinc levels in comparison to the other groups, indicating that disodium EDTA significantly enhanced iron and zinc absorption.

In a follow-up study by Davidsson *et al*. (1998), the influence of ferric sodium EDTA on the absorption and urinary excretion of manganese in 10 healthy adult volunteers (1 male and 9 females) was investigated. The subjects were administered weaning cereals (based on white wheat flour and soy) fortified with 100 mg iron/kg dry cereal product as ferric sodium EDTA or $FeSO_4$ for a period of approximately 30 days. Subsequently, the subjects were provided with cereals containing the alternative iron compound for a second study period of 30 days. All of the test meals were extrinsically labelled with ^{54}Mn as $^{54}MnCl_2$; however, test meals fortified with $FeSO_4$ also contained added ascorbic acid at an iron to ascorbic acid dry weight ratio of 1:5. For control, pre-intervention blood samples were collected from each of the subjects to determine iron-, as well as manganese-status indices. Whole-body retention measurements, as well as urinary samples were obtained to evaluate such parameters as haemoglobin levels, serum iron, TIBC, serum ferritin, and whole blood manganese. The manganese, iron and phytic acid contents of the test cereals were 7.7, 19.7, and 3.9 g/kg dry cereal product, respectively. There was no significant difference in the absorption and urinary excretion of ^{54}Mn with the consumption of ferric sodium EDTA- or $FeSO_4$-fortified

cereals. The authors reported no significant correlation between iron-status indices (*i.e.*, haemoglobin levels, serum iron, TIBC, or serum ferritin) and ^{54}Mn absorption or urinary excretion with consumption of test meals fortified ferric sodium EDTA or $FeSO_4$. Therefore, the authors concluded that the use of ferric sodium EDTA as an iron fortificant in foods would not interfere with the absorption or urinary excretion of manganese, even at an EDTA to manganese molar ratio of 12.7:1.

The apparent absorption of zinc, copper, calcium, and magnesium from test meals fortified with either ferric sodium EDTA or $FeSO_4$ was investigated in 11 healthy infants (5 boys and 6 girls, aged 18 to 27 weeks) (Davidsson *et al*., 2005). Each test meal, which consisted of 20 g of cereal reconstituted with 60 g of hot water, was fed to the infants after an overnight fast, or at least 3 hours after intake of an infant formula on Day 1 of each study. Each study consisted of the ingestion of 2 isotopically labelled test meals (Meals 1 and 2) followed by the collection of faecal material for 72 hours. Capillary blood samples were drawn before and 2 weeks after (*i.e.*, Day 15 of the study) ingestion of Meal 1, and a final blood sample was obtained 2 weeks after intake of the second labelled test meal (*i.e.*, Day 30 of the study). Meal 1 contained stable-isotope labels of iron ($^{58}FeSO_4$ or $^{58}FeCl_3$), 888 µg of zinc ($^{70}ZnCl_2$), and 4.0 mg of calcium ($^{44}CaCl_2$), while Meal 2 contained stable-isotope labels of 1.0 mg of copper ($^{65}CuCl_2$) and 5.0 mg of magnesium ($^{25}MgCl_2$). The total content of added iron, zinc, and calcium was equilibrated in Meal 2 by the addition of minerals with normal isotopic composition. Iron was added to the test meals as 2.0 mg of $^{58}FeSO_4$, or 2.0 mg of $^{58}FeCl_3$ mixed with disodium EDTA, at an iron to EDTA molar ratio of 1:1. Test meals labelled with $^{58}FeSO_4$ also contained added L-ascorbic acid at a molar ratio of iron to ascorbic acid of 1:1.6. The test meals contained 2.0 ± 0.02 mg iron, 1.1 ± 0.01 mg zinc, 358 ± 4 µg copper, 55.6 ± 0.9 mg magnesium, and 45.0 ± 0.3 mg calcium per 100 g of cereal product, before the addition of the stable-isotope labels. According to the authors, there were no significant differences in the apparent absorption of zinc, copper, calcium, or magnesium between test meals fortified with ferric sodium EDTA and $FeSO_4$ plus ascorbic acid. Apparent absorption of zinc and copper from infant cereals fortified with ferric sodium EDTA was 20.5 ± 3.9% and 8.9 ± 3.0%, respectively, compared to 21.1 ± 4.7% and 11.1 ± 6.2%, respectively, for cereals fortified with $FeSO_4$ plus ascorbic acid. The corresponding absorption values for calcium and magnesium were 50.6 ± 6.9 and 47.9 ± 6.1%, respectively, for cereals fortified with ferric sodium EDTA, and 50.0 ± 5.5% and 49.6 ± 7.4%, respectively, for cereals fortified with $FeSO_4$ plus ascorbic acid.

Thirteen (13) healthy, non-pregnant, adult women (aged 20 to 31 years) were provided with 1 of 4 food products at 7-day intervals to evaluate the effect of a novel fortificant mixture on iron and zinc absorption from a dry food supplement designed for pre-school children (Mendoza *et al*., 2004). Food products consisted of a standard or novel food product with either low (192 mg) or high (200 mg) calcium added as $Ca_3(PO_4)_2$. Standard food products contained 10 mg of iron as $FeSO_4$, 10 mg of zinc as $ZnSO_4$, and 50 mg of ascorbic acid per 90 g of food, while novel food products contained 10 mg of iron as ferric sodium EDTA, 10 mg of zinc as zinc methionine, 100 mg ascorbic acid, and 1 g of citric acid. The composition of the experimental diets is summarised in Table 3.1.1-12. Fasting blood

samples were collected on Days 1 and 15 of the study, and absorption of iron and zinc were determined before and immediately after each test food product was consumed using a whole-body counter. The counts were repeated 7 days later to determine absorption of the previously consumed dose. These measurements also served as the baseline values for the next food product. The same procedure was followed for the remaining test food products, which were fed on Days 15 and 22 of the study. The last blood draw and whole-body counting took place on Day 29 of the study. Dietary calcium had no significant effect on iron absorption from these products. Geometric mean iron absorption values were 2.7 and 2.5% of the intake dose for ferric sodium EDTA-fortified foods, and 1.5 and 1.6% of the intake dose for $FeSO_4$-fortified foods with low and high calcium amounts, respectively. Zinc absorption was not significantly affected by the form of zinc consumed; however, a marginal but non-significant association was noted between higher dietary calcium and lower zinc absorption. According to the authors, a mixture of fortificants containing ferric sodium EDTA, $ZnSO_4$ or zinc methionine, ascorbic acid, and citric acid can improve iron and zinc absorption from food products.

3.5 Toxicological Data

3.5.1 Acute Toxicity

Ferric sodium EDTA has been reported to be of low acute oral toxicity with oral LD_{50} values of 2,710 to 10,000 mg/kg body weight (approximately 359 to 1,326 mg iron/kg body weight) in male and female Sprague-Dawley rats, and 794 mg/kg body weight (approximately 105 mg iron/kg body weight) in male and female Kunming mice (Sichuan Provincial Sanitary and Anti-epidemic Station, 1993; Whittaker *et al.*, 2002). Table 3.5.1-1 presents the oral LD_{50} values for ferric sodium EDTA in rats and mice.

Table 3.5.1-1 Reported Oral LD_{50} Values for Ferric Sodium EDTA in Rats and Mice

Species	Sex	LD_{50} (mg/kg bw)	Reference
Sprague-Dawley Rats	Male	10,000	Whittaker *et al*. (2002)
	Male	2,710	Sichuan Provincial Sanitary and Anti-epidemic Station (1993)*
	Female	3,160	
Kunming Mice	Male	794	
	Female	794	

*Tests were conducted according to the "Procedures and Methods for Toxicological Evaluation on Food Safety" of National Standard of the People's Republic of China.

3.5.2 Subchronic Toxicity

3.5.2.1 Subchronic Studies of Ferric Sodium EDTA

For a period of 90 days, groups of Sprague-Dawley rats (10 to 15/sex/group) were administered 0, 10, 40, 160, or 640 mg/kg body weight/day of ferric sodium EDTA in the diet (Sichuan Provincial Sanitary and Anti-epidemic Station, 1993). Parameters examined included body weight, food utilization rate, haematology and blood biochemistry,

histopathology, and organ weights. Treatment with ferric sodium EDTA did not affect the body weight and food utilization rate of the rats. There also was no dose-related effect in haematology parameters. Female rats administered ferric sodium EDTA showed significantly decreased serum creatinine values compared to controls; however, no such effect was noted in their male counterparts. Serum glucose levels of male rats administered 160 or 640 mg/kg body weight/day were significantly lower compared to those of controls. Female rats administered 640 mg/kg body weight/day were reported to have 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 administered 640 mg/kg body weight/day were significantly higher compared to controls; however, these were not observed in female rats. It should be noted that none of these effects was associated with any histopathological alterations 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. Since the effects seen at the highest dose were generally sex-specific, and therefore considered not to be toxicologically significant, a NOAEL of 640 mg/kg body weight/day was determined for ferric sodium EDTA in rats.

In a subsequent 90-day study, ferric sodium EDTA was administered in the diet of groups of Wistar rats (17/sex/group) at a level of 0 (control), 0.31, 1.56, and 3.13% of ferric sodium EDTA, which was reportedly equivalent to 0, 250, 1,250, and 2,500 mg/kg body weight/day, respectively (assuming food intake of 8% of their body weight) (Su *et al.*, 1999). The general condition and food consumption of the animals were evaluated daily throughout the duration of the study. Body weight measurements and food utilization coefficient calculations were performed 1 week before the beginning of the study and every week thereafter. All rats were observed for a period of up to 2 weeks following cessation of ferric sodium EDTA treatment. On Days 45 and 90 of the study, 5 female and 6 male rats from the control, low- (250 mg/kg body weight/day), mid- (1,250 mg/kg body weight/day), and high-dose (2,500 mg/kg body weight/day) groups were killed for measurement of haematology and blood biochemistry values, as well as for organ weight measurements and histopathology examination (*i.e.,* heart, liver, spleen, lung, kidney, stomach, and testis or ovary). Within 2 to 3 days of feeding, a few of the high-dose rats exhibited slight inactivity and retarded body weight gain, and both mid- and high-dose rats exhibited watery stools. While no rats died in the control, low- and mid-dose groups, 4 female, 1 male and 1 female high-dose rats died on Weeks 2, 3, and 7 of the study, respectively. The food utilization coefficient of mid- and high-dose male rats were significantly lower on Day 45, but were not significantly different from controls on Study Day (SD) 90. Mean body weights of high-dose male and female rats were significantly decreased compared to controls throughout the duration of the study, but not at 2 weeks post-treatment. On SD 45, there were no significant difference in concentrations of haemoglobin, red blood cells, and white blood cells (lymphocytes and neutrophils) between ferric sodium EDTA-treated and control groups. However, on SD 90, haemoglobin level was significantly lower, and white blood cell count was significantly higher (due to a significant increase in neutrophil count despite a significant decrease in lymphocyte count) in high-dose male and female rats compared to controls. Serum total protein and albumin levels of high-dose rats also were significantly lower on Day 90 relative to controls. The authors attributed

these effects, in part, to an insufficient intake of nutrients by high-dose rats. There were no significant differences in serum transaminase activity, globulin, albumin:globulin ratio, blood urea nitrogen (BUN), glucose, total cholesterol and triglyceride levels between treated and control groups on Days 45 or 90 of the study. On SD 90, significantly increased spleen and stomach weights were noted in high-dose males, while significantly increased spleen and liver weights were observed in high-dose females when compared to the controls. Histopathological examination of high-dose rats on Day 90 revealed necrotic foci and connective tissue hyperplasia of the liver, interstitial pneumonia and inflammatory cell infiltration of the lungs, and necrosis of the intestinal mucosa. Small liver necrosis foci and/or slight liver connective tissue hyperplasia also were observed in mid-dose rats. Based on the results of the study, the no-observed-effect level (NOEL) for ferric sodium EDTA in rats was determined to be 250 mg/kg body weight/day.

While 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, they have limited applicability to the safety assessment of ferric sodium EDTA in humans. These studies were short English translations of the unpublished reports, which were written in Chinese and did not contain sufficient details about methods or results. For instance, the nature of the diet administered to ferric sodium EDTA-treated and control rats was not reported. The precision of the dosing method employed in the Su *et al*. (1999) study could also be called into question, as the authors themselves later acknowledged that the actual intake of ferric sodium EDTA by the rats was at least 20% higher than the designed dosage. The statistical methods used to analyse the data in both studies also were not mentioned; hence, no judgment can be made on the appropriateness of these methods. There also was considerable overlap between the standard deviations (or standard errors) in the measured variables of treated and control groups of rats. It also is unknown if values for treated rats were significantly different from historical controls. In the Su *et al*. (1999) study, the effects observed in the lungs and intestines of high-dose rats were indicative of an underlying illness that may have exaggerated the effects of ferric sodium EDTA in this group. Therefore, the reliability of the studies by Su *et al*. (1999) and the Sichuan Provincial Sanitary and Anti-epidemic Station (1993) in the overall safety assessment of ferric sodium EDTA is highly limited.

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 (Appel *et al*., 2001). For comparison purposes, another 3 groups of rats (40/group, controls) were provided with 35, 70, or 140 mg iron/kg diet as $FeSO_4$. 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 weights, 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 intestines, spleen, stomach, testes, and thymus). The authors

reported no significant differences in the distribution of iron between the test and control diets, with mean daily intakes of 2.81, 5.67, and 11.19 mg iron/kg body weight for ferric sodium EDTA (approximately 21.2, 42.7, and 84.3 mg ferric sodium EDTA/kg body weight, respectively) and 2.84, 5.69, and 11.54 mg iron/kg body weight for $FeSO_4$, for the low-, mid- and high-doses, respectively. A decreasing trend in the mean daily intake of iron/kg body weight in both ferric sodium EDTA and $FeSO_4$ groups with increasing age of rats was observed and attributed to decreasing food consumption in aging rats. There were no significant differences in the mean body weights and overall mean food consumption between the test and control groups. Reported clinical signs included encrustations around the eyes, malocclusion of incisors, encrustations and red discharge from the nose, sniffing, sparsely haired skin, dermal encrustations, and dermal wounds; however, these were randomly distributed among the various groups and were considered not to be compound-related. There also were no compound-related histopathological changes reported in the control or ferric sodium EDTA-treated groups of rats. Evaluation of haematological parameters after 31 days of feeding revealed significantly increased mean corpuscular haemoglobin levels in the high-dose $FeSO_4$ and ferric sodium EDTA groups (11.2 to 11.5 mg iron/kg body weight/day), and the mid-dose ferric sodium EDTA group (5.7 mg iron/kg body weight/day). In addition, a significant increase in the number of eosinophils was observed at this same time period in the high-dose ferric sodium EDTA group in comparison with the mid-dose $FeSO_4$ group and the other ferric sodium EDTA dose groups. No significant differences in red blood cell, 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 $FeSO_4$ group at Day 32, but not at Day 62 of feeding. Significantly lower total plasma protein concentrations were observed in the ferric sodium EDTA groups in comparison to the controls at Day 62 of feeding. The authors also reported significantly lower plasma albumin levels in the mid- and high-dose ferric sodium EDTA groups than the low-dose (2.8 mg iron/kg body weight/day) ferric sodium EDTA group and low- and mid-dose $FeSO_4$ groups; significantly lower calcium levels in the high-dose ferric sodium EDTA group than the low-dose ferric sodium EDTA and low- and mid-dose $FeSO_4$ groups; and significantly decreased sodium concentrations in the high-dose ferric sodium EDTA and $FeSO_4$ groups, compared to the low-dose $FeSO_4$ group at Day 62. The authors attributed the lower plasma calcium levels in the high-dose ferric sodium EDTA group, for the most part, to the concurrent decrease in plasma albumin levels. However, due to the lack of hepatic damage observed in ferric sodium EDTA-treated rats, the decreased plasma levels of calcium were deemed by the authors not to be toxicologically significant. Table 3.5.2.1-1 lists the results obtained from the evaluated haematological and clinical chemistry variables after 31 and 61 days of feeding.

Table 3.5.2.1-1 The Effects on Haematological and Clinical Chemistry Values in Rats Upon 31 and 61 Days of Feeding with $FeSO_4$ and Ferric Sodium EDTA

Iron Source	RBC (mmol/L)	WBC: Eosinophils (%)	ALP (U/L)	Total Protein (g/L)	Albumin (g/L)	Total Bilirubin (mmol/L)	Ca (mol/L)	Na (mmol/L)
31 days of feeding (n = 20)								
$FeSO_4$ (low)	22.5	0.8	163[d]	63	32	0.9[c]	2.65	146[d,e,f]
FeNaEDTA (low)	22.8	0.5[f]	143	63	32	0.9[c]	2.67	145[d]
$FeSO_4$ (mid)	22.7	0.6[f]	160[d]	64	32	2.7[a,b]	2.67	145[d]
FeNaEDTA (mid)	22.8	0.8	128[a,c,e]	63	32	1.7	2.68	144[a,b,c]
$FeSO_4$ (high)	23.0	0.8	159[d]	64	32	1.9	2.69	144[a]
FeNaEDTA (high)	23.1[a]	1.4[b,c]	143	64	32	1.6	2.70	144[a]
61 days of feeding (n = 20)								
$FeSO_4$ (low)	22.7	0.9	96	68[d,f]	34[d,f]	1.8	2.71[f]	144[e,f]
FeNaEDTA (low)	22.7	1.0	104	66[a]	33[d,f]	1.7	2.67[f]	144
$FeSO_4$ (mid)	22.6	1.2	93	66[f]	33[d,f]	1.6	2.68[f]	143
FeNaEDTA (mid)	22.8	1.2	93	65[a]	31[a,b,c]	1.5	2.65	143
$FeSO_4$ (high)	22.8	1.4	96	67[f]	33	1.6	2.65	143[a]
FeNaEDTA (high)	22.9	1.0	93	64[a,c,e]	31[a,b,c]	1.9	2.60[a,b,c]	143[a]

Appel *et al*. (2001)
RBC – red blood cells; WBC – white blood cells; ALP – alkaline phosphatase; Ca – calcium; Na – sodium
Statistically different ($P < 0.05$) from: [a]$FeSO_4$ (low), [b]ferric sodium EDTA (low), [c]$FeSO_4$ (mid), [d]ferric sodium EDTA (mid), [e]$FeSO_4$ (high), [f]ferric sodium EDTA (high)

The authors reported no significant differences between groups in the absolute and relative organ weights of rats after 31 days of feeding. However, after 61 days, the absolute and relative thymus weights of the low-dose ferric sodium EDTA group were significantly greater than those in the low-dose $FeSO_4$ and the remaining ferric sodium EDTA groups. After 31 days of feeding, plasma concentrations of chloride were reported to significantly decrease with increasing iron levels in both the ferric sodium EDTA and $FeSO_4$ diets. No such trend was observed at the end of 61 days of feeding. In general, plasma triglyceride levels increased with time (*i.e.*, higher after 61 days than after 31 days of feeding), which could be attributed to the natural growth of rats in the study. There was no significant difference between groups in the plasma triglyceride levels throughout the duration of the study, with the exception of significantly decreased levels in the low-dose $FeSO_4$ group at Day 31 of feeding, compared to the high-dose ferric sodium EDTA group. This difference was no longer present at Day 61 of feeding. A significantly higher level of iron in the plasma was observed in rats in the low-dose ferric sodium EDTA group in comparison to those in the mid-dose ferric sodium EDTA group at Day 31; however, there was no significant difference between groups at Day 61. Total iron binding capacity (TIBC) in the blood plasma was not

significantly different between groups at Day 31; however, at Day 61, TIBC was significantly higher in the low-dose $FeSO_4$ group in comparison to all other groups, while the mid-dose $FeSO_4$ and low-dose ferric sodium EDTA groups were reported to have significantly increased TIBC compared to the high-dose ferric sodium EDTA group. Table 3.5.2.1-2 lists the absolute and relative thymus weights and mean levels of triglyceride, TIBC, iron, and chloride in the plasma of rats after exposure to $FeSO_4$ and ferric sodium EDTA in the diet for 31 and 61 days.

Table 3.5.2.1-2 The Effects of 31 and 61 Days of Feeding with $FeSO_4$ and Ferric Sodium EDTA on Thymus Weights, Mean Plasma Levels of Triglyceride, TIBC, Iron, and Chloride in Rats

Iron Source	Triglyceride (mmol/L)	TIBC (mmol/L)	Iron (mmol/L)	Cl (mmol/L)	Absolute Thymus Weight (g)	Relative Thymus Weight (g)
31 days of feeding (n = 20)						
$FeSO_4$ (low)	0.52[f]	91.5	24.9	108[ef]	0.637	1.90
FeNaEDTA (low)	0.72	91.9	28.8[d]	108[ef]	0.699	1.98
$FeSO_4$ (mid)	0.64	97.3	21.7	107	0.669	1.92
FeNaEDTA (mid)	0.60	91.6	20.6[b]	107	0.636	1.85
$FeSO_4$ (high)	0.72	94.0	22.4	105[a,b]	0.646	1.86
FeNaEDTA (high)	0.79	90.1	27.2	106[a,b]	0.688	1.97
61 days of feeding (n = 20						
$FeSO_4$ (low)	1.07	99.6[b,c,d,e,f]	23.1	103	0.533	1.19
FeNaEDTA (low)	1.03	91.5[a,f]	21.5	103	0.623[a,d,f]	1.40[a,d,f]
$FeSO_4$ (mid)	1.01	90.9[a,f]	22.5	103	0.567	1.25
FeNaEDTA (mid)	1.03	90.3[a]	22.3	103	0.523[b]	1.20[b]
$FeSO_4$ (high)	0.96	90.3[a]	22.8	102	0.582	1.32
FeNaEDTA (high)	0.98	84.9[c]	23.5	103	0.531[b]	1.20[b]

Appel *et al*. (2001)
TIBC – total iron binding capacity; Cl – chloride
Statistically different ($P < 0.05$) from: [a]$FeSO_4$ (low), [b]ferric sodium EDTA (low), [c]$FeSO_4$ (mid), [d]ferric sodium EDTA (mid), [e]$FeSO_4$ (high), [f]ferric sodium EDTA (high)

The concentrations of non-haem iron in the liver, spleen and kidney of rats after 31 and 61 days of feeding are summarised in Table 3.5.2.1-3. In general, levels of non-haem iron in the liver, spleen and kidney of rats were reported to increase with increasing iron concentrations in the diet. However, rats fed with ferric sodium EDTA were reported to have significantly lower non-haem iron levels in the liver and spleen than those fed with $FeSO_4$, regardless of the amount of iron in the diet. On the other hand, there was no significant difference in the levels of non-haem iron identified in the kidneys of both control and ferric sodium EDTA-treated rats. The authors concluded that dietary treatment with $FeSO_4$ or ferric sodium EDTA, providing iron at levels of up to 11.5 mg/kg body weight/day, does not result in iron overloading, nor any other toxicologically significant effects in rats. Based on the results of this study, JECFA (2000) concluded that "there was no evidence that administration of iron in the form of sodium iron EDTA would result in greater uptake of iron

than from an equivalent dietary concentration of $FeSO_4$ once the nutritional requirement for iron is satisfied." JECFA concluded that the use of ferric sodium EDTA would be safe for use in food fortification programs that would provide 0.2 mg iron/kg body weight/day. The NOAEL was considered to be 84.3 mg ferric sodium EDTA/kg body weight/day, providing 11.19 mg iron/kg body weight/day.

Table 3.5.2.1-3 Reported Concentrations of Non-Haem Iron in the Liver, Spleen and Kidney of Rats After 31 and 61 Days of Feeding with $FeSO_4$ and Ferric Sodium EDTA

Iron Source	Non-haem Iron Concentration (mg/g tissue)		
	Liver	Spleen	Kidney
31 days of feeding (n = 20)			
$FeSO_4$ (low)	67[c,e,f]	171[e]	29[f]
FeNaEDTA (low)	62[c,d,e,f]	158[e]	26[e,f]
$FeSO_4$ (mid)	89[a,b,e]	213[e]	30[f]
FeNaEDTA (mid)	78[b,e]	201[e]	30[f]
$FeSO_4$ (high)	116[a,b,c,d,f]	286[a,b,c,d,f]	32[b,f]
FeNaEDTA (high)	91[a,b,e]	217[e]	39[a,b,c,d,e]
61 days of feeding (n = 20)			
$FeSO_4$ (low)	106[e]	1,019	44
FeNaEDTA (low)	96[ef]	892[c,e]	43[c,f]
$FeSO_4$ (mid)	120	1,107[b,d,f]	49[b]
FeNaEDTA (mid)	107[e]	919[c,e]	45
$FeSO_4$ (high)	126[a,b,d]	1,126[b,d,f]	48
FeNaEDTA (high)	119[b]	920[c,e]	49

Appel *et al*. (2001)
Statistically different ($P < 0.05$) from: [a]$FeSO_4$ (low), [b]Ferric sodium EDTA (low), [c]$FeSO_4$ (mid), [d]FeNaEDTA (mid), [e]$FeSO_4$ (high), [f]Ferric sodium EDTA (high)

3.5.2.2 Subchronic Studies of Other Sources of EDTA

Chan (1964) examined the effects of administering 0, 0.5 or 1.0% of calcium disodium EDTA or disodium EDTA (approximately 0, 500, and 1,000 mg/kg body weight/day, respectively) in the diet of groups of 50 Wistar rats (number and sex per group not reported) for a period of 205 days. The animals were evaluated on parameters of growth, clinical signs of toxicity, food consumption, haematology and clinical biochemistry, mortality, dental and skeletal studies, as well as gross histopathological examination of the internal organs and tissues. Food and oxygen consumption rates were not significantly different between rats of the treatment and control groups. With the exception of diarrhoea and anaemic appearance in rats administered 1,000 mg disodium EDTA/kg body weight/day, no clinical signs of toxicity were observed in any of the groups of rats. Male rats in the high-dose disodium EDTA group and female rats in the high-dose calcium disodium EDTA group (1,000 mg/kg body weight/day) exhibited a retardation of growth compared to controls; however, there was no significant difference in growth between rats receiving the lower dose of calcium disodium EDTA or disodium EDTA (500 mg/kg body weight/day) and the controls. Significantly lower

white and red blood cell counts also were reported in the high-dose disodium EDTA group compared to the other groups; however, individual values were within the normal range for an adult rat. Serum calcium levels and blood coagulation rates were significantly increased in the high-dose disodium EDTA group compared to controls; the latter observation being attributed by the author to the complexing of the free ionic calcium in the blood. With the exception of the significantly reduced total ash content of the tibia reported in the high-dose disodium EDTA group, no significant differences in total ash content of skeletal bones were noted between treated and control groups. No abnormalities were reported upon gross examination of the major organs and tissues of rats, including the liver, kidney, and spleen; however, high-dose disodium EDTA rats exhibited a slight dilation of the hepatic sinusoids, a slight increase in the number of Kupffer cells, as well as an "indistinct appearance" of the tubular epithelium of the kidneys. Based on the results of this study, NOEL values of 1,000 mg/kg body weight/day for calcium disodium EDTA and 500 mg/kg body weight/day for disodium EDTA were determined in rats.

Groups of 4 mongrel dogs (1 to 3/sex/group) were provided with diets containing 0, 50, 100, and 250 mg/kg body weight/day of calcium disodium EDTA for a period of 12 months (Oser *et al*., 1963). Evaluated parameters included: daily physical condition and behaviour, weekly body weight measurements; 12-week haematological examination, as well as blood sugar and non-protein nitrogen levels; and 12-, 26-, and 52-week urinary sugar and albumin measurements. The animals were killed at the end of the 12 months of study, and histopathological examinations of the liver, kidneys, and pituitary of all of the dogs were conducted. Additionally, the pancreas, stomach, small intestine, colon, spleen, heart, thyroid, lymph nodes, bladder, gonads, adrenals, marrow, and bone of the dogs in the high-dose group (250 mg calcium disodium EDTA/kg body weight/day) were histopathologically examined. According to the authors, the low-, mid-, and high-dose groups actually consumed 58, 130, and 338 mg/kg body weight/day of calcium disodium EDTA, respectively. There were no significant differences in weight gain, urine or blood chemistry values, haematological parameters, and organ weights (liver, kidneys, spleen, heart, adrenals, and gonads) in any of the calcium disodium EDTA-treated groups. In addition, examination of the tibias of each dog revealed no evidence of osteoporosis or other osseous changes. No significant gross pathological abnormalities or histopathological lesions were observed in any of the organs examined in any group. Based on the results of this study, the NOEL for calcium disodium EDTA was considered to be 250 mg/kg body weight/day, which was the highest dose tested in the study.

3.5.3 Mutagenic and Genetic Toxicity

Ferric sodium EDTA has been tested in *in vitro* short-term mutagenicity tests; the results of which are presented in Table 3.5.3-1. There was no evidence of mutagenicity in the Ames assay (plate incorporation and pre-incubation methodologies) when *Salmonella typhimurium* strains TA97a, TA98, TA100, TA102, TA1535, TA1537, and TA1538 were incubated with up to 10,000 µg/plate of iron as ferric sodium EDTA, with or without metabolic activation (Sichuan Provincial Sanitary and Anti-epidemic Station, 1993; Dunkel *et al*., 1999). Negative results also were reported when up to 5,000 µg/plate of ferric sodium EDTA was evaluated

in the WP2 Mutoxitest conducted with *Escherichia coli* strains IC188 and IC203 without metabolic activation (Martínez *et al*., 2000). Dietary administration of up to 400 mg/kg body weight of ferric sodium EDTA produced negative results in an *in vivo* micronucleus assay in mouse bone marrow cells (Sichuan Provincial Sanitary and Anti-epidemic Station, 1993).

In the mouse lymphoma assay of L5178Y cells, ferric sodium EDTA was tested at concentrations providing 1.3, 2.6, 162.5, and 325.0 µg/mL of iron in the absence of metabolic activation, and at concentrations providing 0.026, 0.052, 1.625, 3.250, and 6.500 µg/mL of iron in the presence of metabolic activation (Dunkel *et al*., 1999). In the absence of metabolic activation, the increase in mutation frequency was noted to be more than double that of the negative control only at the highest dose of 325.0 µg iron/mL, which produced cytotoxicity, with a relative total growth (RTG) of 33.5% of control. In the presence of metabolic activation, dose-dependent increases in mutation frequency (=2-fold that of the negative control) were observed at the 3 highest concentrations tested (1.625, 3.250, and 6.500 µg iron/mL), which resulted in RTG percentages of 53.0, 38.0, and 19.0% of control, respectively. The authors attributed these observations to the ferric component of ferric sodium EDTA, since disodium EDTA produced negative results in the mouse lymphoma assay with or without metabolic activation.

Moreover, similar results were observed in the mouse lymphoma assay of other ferrous or ferric salts (Dunkel *et al*., 1999). In the presence of metabolic activation, $FeSO_4$ produced dose-dependent increases in mutation frequency (=2-fold that of the negative control) at all doses tested, with the exception of the lowest dose (1.005, 1.206, and 1.508 µg iron/mL); however, these also were accompanied by reductions in cell viability, with RTG percentages of 54.5, 41.5, and 5.0% of control, respectively. Ferrous fumarate also produced dose-dependent increases in mutation frequency at all dose levels tested in the absence of metabolic activation, with the exception of the lowest dose. However, reductions in cell viability (RTG percentages of 64.5 and 15.0% of controls) occurred only at the 2 highest doses tested (309.3 and 315.9 µg iron/mL, respectively). While the RTG of cells incubated with $FeCl_3$, in the presence of metabolic activation, reached a minimum of 66.0% at the highest dose tested, dose-dependent increases in mutation frequency (>2-fold that of the negative control) occurred at the 3 highest doses tested (0.824, 1.030, and 1.236 µg iron/mL).

In a review of the results reported by Dunkel *et al.* (1999), Heimbach *et al.* (2000) concluded that mouse lymphoma L5178Y cells may be "particularly to sensitive incorporation of excessive quantities of iron salts in the tissue culture growth medium." It also has been suggested that the significance of mutations produced by iron compounds, added at non-physiological concentrations, in an *in vitro* screening system, is difficult to extrapolate for relevance to intact organisms (Heimbach *et al*., 2000).

Table 3.5.3-1 Summary of *In Vitro* Mutagenicity Assays of Ferric Sodium EDTA				
Test System	**Test/Assay**	**Concentration**	**Results**	**Reference**
In Vitro				
Salmonella typhimurium TA97a, TA98, TA100, TA102, TA1535, TA1537, and TA1538	Ames	Up to 10,000 µg iron/plate	Negative (±S9)[1]	Dunkel *et al*. (1999)
S. typhimurium TA97, TA98, TA100, and TA102	Ames	200, 1,000, or 5,000 µg/plate	Negative (-S9[2])	Sichuan Provincial Sanitary and Anti-epidemic Station (1993)
S. typhimurium TA97, TA98, TA100, TA102	Ames	40, 200, 1,000, or 5,000 µg/plate	Negative (+S9[3])	Sichuan Provincial Sanitary and Anti-epidemic Station (1993)
Escherichia coli IC188 and IC203	WP2 Mutoxitest	Up to 5,000 µg per plate	Negative (-S9)	Martínez *et al*. (2000)
L5178Y $TK^{+/-}$ cells	Mouse lymphoma	1.3, 2.6, 162.5, or 325.0 µg iron/mL	Negative[4] (-S9)	Dunkel *et al*. (1999)
L5178Y $TK^{+/-}$ cells	Mouse lymphoma	0.026, 0.052, 1.625, 3.250, or 6.500 µg iron/mL	Positive[5] (+S9)	Dunkel *et al*. (1999)
In Vivo				
Bone marrow of male and female Kunming mice	Micronucleus Induction	50, 100, 200, or 400 mg/kg bw[6]	Negative	Sichuan Provincial Sanitary and Anti-epidemic Station (1993)

[1] With or without metabolic activation
[2] Without metabolic activation
[3] With metabolic activation
[4] Increase in mutation frequency was =2-fold the negative control only at the highest dose tested, which produced significant cytotoxicity, with a relative total growth of 33.5% of control.
[5] Increases in mutation frequency were =2-fold the negative control only at the 3 highest doses tested (1.625, 3.250, and 6.500 µg iron/mL), which produced significant reductions in cell viability, with relative total growth percentages of 53.0, 38.0, and 19.0% of control, respectively.
[6] Administered in the diet; evaluation of micronucleus formation conducted 30 hours later.

3.5.4 Chronic Toxicity and Carcinogenicity

No chronic carcinogenicity studies have been conducted with ferric sodium EDTA; however, several studies have been conducted with other EDTA salts, which are summarised in the following paragraphs.

Groups of F344 rats (50/sex/group) were administered 3,750 or 7,500 mg/kg of trisodium EDTA·$3H_2O$ in the diet for a period of 103 weeks (approximately 187.5 and 375 mg/kg body weight/day, respectively) (NCI, 1977). A control group of 20 rats/sex received a basal diet. The animals were evaluated daily for clinical signs of toxicity, weighed at regular intervals, and killed at the end of the study period for gross and microscopic examination of all of the major tissues and organs. No significant differences between the trisodium EDTA-treated and control groups in average body weight and clinical signs of toxicity were reported. There was a negative dose-related trend in survival reported for all of the rats in the study; however, this trend was significant only among female trisodium EDTA-treated rats. Gross

and histopathological examinations of all major tissues and organs revealed no significant differences between trisodium EDTA-treated and control groups of rats in the nature, incidence, and severity of tumours. The authors concluded that trisodium EDTA, administered in the diet at levels of 3,750 and 7,500 mg/kg, was not demonstrated to be carcinogenic in F344 rats. Based on the results of this study, the NOEL was considered to be 7,500 mg/kg trisodium EDTA·$3H_2O$ (approximately 375 mg/kg body weight/day), the highest dose tested.

Trisodium EDTA·$3H_2O$ was administered in the diet of groups of B6C3F_1 mice (50/sex/group) at levels of 3,750 or 7,500 mg/kg for a period of 103 weeks (approximately 562.5 and 1,125 mg/kg body weight/day) (NCI, 1977). For control, another group of mice (20/sex/group) received a basal diet (containing no trisodium EDTA) for 104 weeks. The animals were evaluated daily for clinical signs of toxicity, weighed at regular intervals, and necropsied at the end of the study period for gross and microscopic examination of all of the major tissues and organs. Mean body weights were lower in male mice of the high-dose group (1,125 mg/kg body weight/day of trisodium EDTA) compared to controls, while a dose-related decrease in mean body weights was reported in trisodium EDTA-treated female mice; however, the statistical significance of these results was not reported. The presence of ascites also was reported in trisodium EDTA-treated mice during the second year of the study. There was no significant difference in survival rates between trisodium EDTA-treated and control groups of mice. Similar to the results observed in F344 rats, gross and histopathological examinations of all major tissues and organs revealed no significant differences between the trisodium EDTA-treated and control mice in the nature, incidence, and severity of tumours. The authors concluded that trisodium EDTA, administered in the diet at levels of 3,750 and 7,500 mg/kg, was not demonstrated to be carcinogenic in B6C3F_1 mice. Based on the results of this study, the NOEL was considered to be 7,500 mg/kg trisodium EDTA (approximately 1,125 mg/kg body weight/day), the highest dose tested.

Oser *et al*. (1963) studied the effects of administering 0, 50, 125, or 250 mg/kg body weight/day of calcium disodium EDTA 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, and their body weights and food intakes were measured weekly. Food utilization efficiency was measured at 12 weeks, while blood haemoglobin levels, red and white blood cell counts, differential white cell counts, prothrombin time, blood sugar and non-protein nitrogen, and serum calcium levels were measured at Weeks 6 and 12, and at Months 12, 18, and 24. Additionally, 2 rats/sex/group were killed at 12 weeks for histopathological examination. Ten rats/group were permitted to mate after approximately 13 weeks of study for the reproductive and developmental toxicity study (see Section 3.5.4). There were no significant differences in physical appearance or behaviour reported among any of the groups of rats. No adverse effects on growth were noted in low- (50 mg/kg body weight/day), mid- (125 mg/kg body weight/day), and high-dose (250 mg/kg body weight/day) groups of rats. With the exception of significantly increased body weight gains in mid- and high-dose female rats, there were no significant differences in body weight gains between the control and calcium disodium EDTA-treated groups. Levels of haemoglobin, haematocrit, blood sugar and non-protein

nitrogen, urinary albumin and sugar, red and white blood cell counts, and prothrombin times were not significantly different among the various dose groups. The authors reported squamous or round epithelial cells in the calcium disodium EDTA-treated rats; however, the occurrence of these observations was not significantly different from controls. Non-dose-related deaths occurred in 1 low-dose male at Week 12, 1 mid-dose female at Week 8, and 3 control males between Weeks 10 and 12. Based on the results of this study, the NOEL was considered to be 250 mg calcium disodium EDTA/kg body weight/day, the highest dose tested. This study was used by JECFA to assign an ADI for EDTA of 2.5 mg/kg body weight/day or 150 mg/person/day, calculated as calcium disodium EDTA (JECFA, 1966, 1974).

A total of 33 Wistar rats (number and sex per group not reported) were divided into 5 groups and dosed with 0, 0.5, 1.0, or 5.0% disodium EDTA in the diet for a period of 2 years (approximately 0, 250, 500, and 2,500 mg/kg body weight/day, respectively) (Yang, 1964). The animals were evaluated on parameters of growth, food consumption, haematology, mortality, ash content of bones and gross examination of internal organs and tissues. No significant differences were noted between treated and control on parameters of growth, food intake, haematology (*i.e.*, blood coagulation times and red blood cell counts), and ash content of the tibia and femur. An unspecified number of deaths occurred in both control and low- (250 mg/kg body weight/day) and mid-dose (500 mg/kg body weight/day) groups; however, the deaths were attributed to pneumonia and not to the compound administered. The highest mortality rate was reported to occur in the control group, while no deaths were reported for the high-dose group (2,500 mg/kg body weight/day). No gross lesions were observed upon histopathological examination of the internal organs and tissues, including the heart, liver, pancreas, kidneys, urinary bladder, stomach, small and large intestines, lungs, spleen, ovaries, testes, voluntary muscle, and bone marrow smears. Based on the results of this study, the NOEL was considered to be 5% disodium EDTA (approximately 2,500 mg/kg body weight/day) in rats.

3.5.5 Reproduction and Developmental Toxicity

3.5.5.1 Reproductive and Developmental Studies of Ferric Sodium EDTA

Groups of female Sprague-Dawley rats (10 or 11/group) were administered 0, 50, 200, or 800 mg/kg body weight of ferric sodium EDTA by gavage on Days 7 to 16 of gestation (Sichuan Provincial Sanitary and Anti-epidemic Station, 1993). The rats were killed on Day 20 of gestation and necropsied. Significantly decreased body weights on Days 16 and 20 of gestation were reported in high-dose (800 mg/kg body weight/day) dams; however, no significant differences in maternal body weights were observed between control and low- (50 mg/kg body weight/day) or mid-dose (200 mg/kg body weight/day) dams. Mid- and high-dose dams gave rise to foetuses with slightly reduced body weights and heights relative to those of controls. Fontanel sizes tended to be higher in foetuses of the mid- (16.36 ± 3.89 mm^2) and high-dose (15.84 ± 4.41 mm^2) groups compared to those in the control (13.07 ± 3.87 mm^2) or low-dose (13.85 ± 3.87 mm^2) groups; however, a clear dose-response relationship was not observed. There was no significant difference in the incidence of foetal

resorption between ferric sodium EDTA-treated and control groups. The incidence of foetal mortality was not dose-related, with deaths occurring in 11/133 (8.3%), 0/117 (0%), and 9/123 (7.3%) of the foetuses in the low-, mid-, and high-dose groups, respectively. It should be noted that all of the dead foetuses were produced by only 1 of 11 dams (9.1%) in each of the low- and high-dose groups. External examination of rats revealed no malformations in any of the treated or control groups. While the overall incidence of delayed ossification and/or sternum fusion was reported to be significantly higher in high-dose foetuses (67.3%), it should be noted that lower incidences were observed in low- (10.3%) and mid-dose (28.3%) foetuses compared to those of controls (32.3%). Hence, these effects may be classified as variations or deviations, not aberrations or malformations, given that they were not associated with alterations in external morphology or foetal survival, and that they occurred at a relatively high incidence in the control group (Fritz and Hess, 1970; Kimmel and Wilson, 1973; Khera, 1981; Solecki *et al*., 2001). Furthermore, these skeletal effects (*i.e.*, increased fontanel sizes, delayed ossification and/or sternum fusion) may have occurred secondary to foetal immaturity or growth retardation, which was reflected by the low foetal weights and heights, as well as to the decreased body weights of the dams (Khera, 1981; Ariyuki *et al*., 1982). Therefore, these effects are not indicative of a direct teratogenic effect of ferric sodium EDTA. Based on the results of the study, ferric sodium EDTA was determined to have a NOEL value of 200 mg/kg body weight/day for maternal and developmental toxicity in rats.

While the Sichuan Provincial Sanitary and Anti-epidemic Station (1993) is the only developmental toxicity study available on ferric sodium EDTA, it has limited applicability to the safety assessment of ferric sodium EDTA in humans. As previously mentioned, this study was a short English translation of the unpublished report, which was written in Chinese and did not contain sufficient details about methods or results. For instance, the nature of the diet administered to ferric sodium EDTA-treated and control rats was not reported. The methods by which the dams were impregnated, examined for pregnancy, and subsequently chosen for the study also were unclear. The number of dams per group was unsatisfactory, with the utilization of only 10 to 11 dams/group as opposed to 16 to 20 dams/group according to international developmental toxicity test guidelines. Furthermore, it was not reported whether the dams were observed for clinical signs of toxicity; hence, it is unknown if the dams experienced diarrhoea, which could have accounted for the body weight effects observed. There also was no mention of other important end-points in the study, including maternal food consumption, maternal gravid uterine weight, maternal necropsy findings, corpora lutea, implantation sites, pre-implantation loss, and post-implantation loss (early or late resorption). The statistical methods used to analyse the data also were not discussed; therefore, no judgment can be made on the appropriateness of these methods. There is considerable overlap between the standard deviations (or standard errors) in the measured variables of treated and control groups of rats. Moreover, it is unknown if the values observed for the ferric sodium EDTA-treated rats were significantly different from historical controls. Based on these factors, the reliability of the developmental toxicity study by the Sichuan Provincial Sanitary and Anti-epidemic Station (1993) in the overall safety assessment of ferric sodium EDTA is highly limited.

Ferric sodium EDTA produced negative results in a sperm cell malformation test conducted in groups of 8-week-old male Kunming mice (6/group) administered 100, 200, or 400 mg ferric sodium EDTA/kg body weight/day in the diet for a period of 5 days (Sichuan Provincial Sanitary and Anti-epidemic Station, 1993).

3.5.5.2 Reproductive and Developmental Studies of Other Sources of EDTA

In a study by Oser *et al.* (1963), which is described in detail in Section 3.5.4, 4 successive generations of Wistar rats were exposed to 0, 50, 125, or 250 mg/kg body weight/day of calcium disodium EDTA in the diet, and terminal observation of rats receiving the test diets for 0, 6, 12, 18, or 24 months were obtained from the F_3, F_2, F_1, and F_0 generations, respectively. No adverse effects on the growth of 4 generations of calcium disodium EDTA-treated rats were reported. Weight gain differences observed among the different generations of rats were not related to dose. There were no significant differences in the levels of haemoglobin, haematocrit, blood sugar and non-protein nitrogen, urinary albumin and sugar, red and white blood cell counts, and prothrombin times observed among any of the generations of rats. No deaths were reported in any of the descendant generations of rats receiving the highest dose of 250 mg calcium disodium EDTA/kg body weight/day. Additionally, no reproductive or teratogenic effects were observed in any of the 3 generations of offspring. The NOEL was considered to be 250 mg calcium disodium EDTA/kg body weight/day.

Groups of Wistar rats (2 males and 4 females/group) were maintained on diets containing 0, 0.5, 1.0, and 5.0% disodium EDTA (approximately 0, 250, 500, and 2,500 mg/kg body weight/day, respectively), and allowed to mate upon reaching the age of 100 days (Yang, 1964). In order to obtain second litters, mating was repeated 10 days after weaning of the first litters. Normal first and second litters were reported from rats administered 0, 0.5, and 1.0% of disodium EDTA; however, the high-dose group of rats (2,500 mg/kg body weight/day) did not to produce any litters, even after 2 months of mating. This study is further described in Section 3.5.4. The NOEL for reproductive effects was considered to be 500 mg disodium EDTA/kg body weight/day.

Kimmel (1977) investigated the effect of route of administration of disodium EDTA in 4 groups of pregnant CD rats (8 to 42/group). Rats were administered disodium EDTA on Days 7 through 14 of gestation, at a concentration of 3% in the diet (approximately 954 mg disodium EDTA/kg body weight/day), by gastric intubation twice daily with 625 or 750 mg disodium EDTA/kg body weight (total of 1,250 and 1,500 mg disodium EDTA/kg body weight/day, respectively), or by subcutaneous injection of 375 mg disodium EDTA/kg body weight/day. For control, 3 groups of rats (14 to 38/group) received semi-purified diets without disodium EDTA, gastric intubation twice daily with a potassium phosphate buffer, or subcutaneous injection of the phosphate buffer. The dams were observed daily for weight changes, general physical condition and food consumption until they were killed at Day 21 of gestation, whereby their reproductive statuses, including number of implantation sites, foetal weights and malformations, were examined. Maternal body weight gain was significantly decreased in all disodium EDTA-treated groups, with significant body weight losses

observed in dams provided with disodium EDTA in the diet or by subcutaneous injection. The authors attributed the decreased body weight gain to decreased food consumption and diarrhoea in these groups. Foetal weights were significantly decreased in rats administered disodium EDTA subcutaneously or in the diet, while no significant difference in foetal weight was reported between controls and those administered disodium EDTA by gastric intubation. There were no deaths in any of the dams that received 954 mg/kg body weight/day of disodium EDTA in the diet; however, foetal resorptions (33%) and malformations (71%) were significantly higher in this group compared to controls. Dams administered 1,250 mg/kg body weight/day of disodium EDTA by gastric intubation had 8/22 (36%) deaths and significantly increased foetal malformations (20%); however, no significant effects on foetal resorptions (7%) were observed relative to controls. Administration of 1,500 mg/kg body weight/day of disodium EDTA by gastric intubation resulted in 7/8 (88%) maternal deaths. Subcutaneous injection of 375 mg disodium EDTA/kg body weight/day was reported to produce 6/25 (24%) maternal deaths and significantly higher foetal resorptions (32%) than controls; however, the frequency of foetal malformations was not significantly different from controls. The authors suggested that the differences in toxicity and teratogenicity reported for the different routes of exposure used in this study probably relate to absorption differences, interaction with metals and stress associated with administration of the compounds. Therefore, the authors concluded that the route of exposure to disodium EDTA is an essential factor in determining its potential for lethal or teratogenic effects.

In a follow-up study conducted by Schardein *et al*. (1981), equimolar amounts of EDTA and its salts (disodium EDTA, trisodium EDTA, calcium disodium EDTA, and tetrasodium EDTA) were administered orally by gastric intubation to groups of female CD rats (17 to 20/group) from Gestation Days (GD) 7 to 14. The actual dosages administered to the rats in 2 equally divided doses per day were 967, 1,243, 1,245, 1,340, 1,374 mg/kg body weight of EDTA, disodium EDTA, trisodium EDTA, calcium disodium EDTA, and tetrasodium EDTA, respectively. For control, a group of rats was treated with phosphate buffer orally by gavage, while another group remained untreated. All of the rats were observed during the post-treatment period of Days 14 to 21 of gestation, prior to being killed on GD 21, whereby all of the foetuses were subjected to gross examination. Significant compound-related effects in the EDTA-treated groups included diarrhoea and depressed activity. Mortality was reported in 3 of the dams treated with disodium EDTA; however, necropsy revealed no gross abnormalities. The authors attributed the deaths of 2 dams treated with trisodium EDTA and EDTA to errors in dosing. Food intake was slightly decreased in the EDTA-treated groups during the treatment period, but was comparable to, or slightly greater than, the controls over the post-treatment period. Significantly decreased body weight gains were observed in all EDTA-treated groups compared to controls; however, recovery was evident in all of the groups by the post-treatment period. There were no significant differences in litter size, sex ratio, foetal weights, and foetal mortality between the EDTA-treated and controls. The authors reported no teratogenic effects due to EDTA treatment, and concluded that EDTA and its salts have little or no propensity for teratogenicity when administered orally to rats. The NOAELs were considered to be 967, 1,243, 1,245, 1,340, 1,374 mg/kg body weight of

EDTA, disodium EDTA, trisodium EDTA, calcium disodium EDTA, and tetrasodium EDTA, respectively.

3.5.6 Fortification Studies of Ferric Sodium EDTA in Humans

Ferric sodium EDTA has been used in numerous field trials in developing countries for the iron fortification of foods; some of these studies are summarised in Table 3.5.6-1. 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 (0.07 to 0.25 mg iron/kg body weight/day) (Garby and Areekul, 1974; Ballot *et al*., 1989a; Viteri *et al*., 1995; Huo *et al.*, 2002; Thuy *et al.*, 2003). Remarkable improvements in iron status indicators, including serum levels of haemoglobin and ferritin, FEP, TIBC, and iron stores, were reported in the fortified groups compared to the controls (Garby and Areekul, 1974; Ballot *et al*., 1989a; 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*., 1989a; 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).

Overall, the results of these studies support the safe intake of ferric sodium EDTA by humans. As discussed in Section 2.7.2, the highest estimated mean and 95th percentile intakes of iron from typical example fortified food-uses of ferric sodium EDTA in the E.U. are 4.93 and 13.28 mg/person/day, respectively. The field trials described above indicate that similar intake levels (up to 15 mg iron/person/day) are well tolerated and without adverse effects, thus supporting the safe consumption of ferric sodium EDTA at the intended levels of use.

Table 3.5.6-1 Summary of Field and Clinical Trials Using Ferric Sodium EDTA to Fortify Various Food Vehicles

	Garby and Areekul (1974)	**Ballot *et al.* (1989a)**	**Viteri *et al.* (1995)**	**Huo *et al.* (2002)**	**Thuy *et al.* (2003)**
Geographical region	Thailand	South Africa	Guatemala	China	Vietnam
Population studied	2 rural villages	Urban Indian community in a municipal housing estate	4 rural communities (2 lowlands and 2 highlands)	Children aged 11 to 17 years	Adult women
Design of trial	Controlled (one village); not blinded	Controlled (random allocation by families); double-blinded	Controlled; double-blinded	Controlled, double-blinded, randomized	Controlled, double-blinded, randomized
Sample population	Test village (n=284); Control village (n=330)	263 families (n=672); 129 control families; 134 fortified families; Hb <9 g/dL excluded	T_1 (n=1,144); T_2 (n=1,756); T_3 (1,645); Control (n=1,095); severely under-nourished children, anaemic, pregnant women, Hb < 90 g/L excluded	Control (n=102), low FeNaEDTA (n=102), high FeNaEDTA (n=100)	Control (n=76), Test (n=76)
Food vehicle	Fish-sauce (salt substitute) 30 g NaCl/L, 10 mg Fe/L distributed by village head-man as required	Masala (curry powder) distributed directly to families monthly; free of charge	Off-white sugar distribution: sold to store keepers; purchased by participants (relatively good compliance)	Soy sauce added to soup served daily at lunch	Fish sauce consumed with meals based on noodles or rice
Level of fortification and intake	1 mg Fe/mL; 10-15 mg Fe/person/day	1.4 mg Fe/g; 7.7 mg Fe/person/day	124 mg Fe/kg; 4.3-4.7 mg Fe/person/day	1 mg Fe/mL; 5 and 20 mg Fe/person/day	1 mg Fe/mL; 10 mg Fe/person/day
Duration of trial	12 months	24 months	32 months	3 months	6 months
Abnormal iron status before trial (%)	30-50 of population anaemic; 34 PCV below normal	IDA 24 (F), 4 (M) ID 53 (F), 24 (M)	27.3 (T_1), 41.7 (T_2), 10.2 (T_3), 13.9 (Control) of population anaemic	100 anaemic (all groups)	100 anaemic (all groups)
Measurements taken	PCV	Haemoglobin, %Sat, serum ferritin	Haemoglobin, %Sat, FEP, serum ferritin, Fe stores	Haemoglobin, serum iron, serum ferritin, transferritin, TIBC	Haemoglobin, serum ferritin, serum transferrin receptor, serum transferrin receptor:serum ferritin ratio

IDA = iron deficiency anaemia; ID = iron deficiency; % Sat = % saturation of transferrin; FEP = free erythrocyte protoporphyrin; PCV = packed cell volume; TIBC = total iron binding capacity

3.5.6.1 Ferric Sodium EDTA-Fortified Fish and Soy Sauces

A 1-year fortification trial was conducted in 2 villages of the Nakorn Nayok Province of Thailand (Garby and Areekul, 1974). Village 6, with a population of 460, served as the test village, while village 4 (population of 420) served as the control village. Subjects from village 6 were provided with Thai fish sauce fortified with 6.56 mg ferric sodium EDTA/mL (equivalent to 1 mg of iron/mL). Daily consumption of the ferric sodium EDTA-fortified fish sauce ranged from 10 to 15 mL/day, (approximately 10 to 15 mg iron/day). Packed red cell volume (PCV) measurements were carried out throughout the duration of the study in 346 (82%) and 413 (90%) of the subjects from villages 4 and 6, respectively, excluding children below the age of 2. A total of 284 (68%) and 330 (70%) subjects from the control and test villages, respectively, completed the study. When compared to baseline values, significantly increased PCV values were noted in children (up to 14 years of age), and male and female adults of the test village at the end of the 1-year fortification period. These results are presented in Table 3.5.6.1-1. Following the 1-year fortification trial, the sub-maximal physical working capacities of 21 male subjects from the test village, as well as 4 male subjects from the control village, were examined using a bicycle ergometer. It was reported that the mean values of work intensity from subjects of the test village significantly increased from 106 W at baseline to 113 W at the end of the study, while no such increase was noted in subjects of the control village. Under the assumption that a change in PCV value of =3 units is significant, the authors suggested that 16% of the subjects in the control village became anaemic, while 20% recovered from anaemia by the end of the fortification period. In the test village, only 9% became anaemic, but 35% of the subjects recovered from anaemia by the end of the study.

Table 3.5.6.1-1 Reported PCV[a] Values in the Control and Test Villages

Subject Population	Number of Subjects	Initial Value	Mean Change	S.E.
Control Village (Village 4)				
Children[b]	126	36.9	-0.02	0.18
Male Adults	66	40.1	-0.02	0.40
Female Adults	92	36.0	+0.22	0.32
Test Village (Village 6)				
Children	117	36.7	+1.38[c]	0.19
Male Adults	98	40.1	+1.45[c]	0.33
Female Adults	115	36.1	+1.56[c]	0.31

Garby and Areekul (1974)
[a] PCV – packed red cell volume
[b] Boys = 14 years; girls = 12 years.
[c] All differences in the mean changes are significant, $p < 0.05$.

Thuy *et al.* (2003) conducted a randomized, double blind, placebo-controlled study of the effect of consumption of ferric sodium EDTA-fortified fish sauce on iron status among anaemic Vietnamese women. Two groups of 76 women consumed meals based on noodles or rice with 10 mL of fish sauce, either unfortified or fortified with 10 mg iron as ferric sodium

EDTA. The meals were consumed 6 days per week for a period of 6 months. The mean iron content of the unfortified and fortified fish sauce was 28.2 and 1,250 mg/L, respectively. Measurements of haemoglobin, serum ferritin, and serum transferrin receptor were conducted at baseline and after 3 and 6 months. There was no significant difference between groups in the prevalence of anaemia at baseline. After 3 and 6 months, haemoglobin and total body iron levels were significantly greater and transferrin receptor and serum transferrin receptor:serum ferritin ratio were significantly decreased in the fortified group in comparison to controls. Similar changes in these parameters were observed within the fortified group after 6 months compared to baseline, while no significant changes were observed in the control group compared to baseline. The prevalence of low serum ferritin, iron deficiency, and iron deficiency anaemia significantly decreased in the fortified group, while no significant changes in iron status or the prevalence of anaemia were observed in the control group.

Similar results were reported in another study conducted in Chinese school children (aged 11 to 17 years) with iron deficiency anaemia (Huo *et al.*, 2002). A total of 304 children were randomized into 3 groups (100 to 102/group) and received a soup containing unfortified soy sauce, or soy sauce fortified with ferric sodium EDTA providing either 5 (low ferric sodium EDTA) or 20 mg (high ferric sodium EDTA) iron/day for a period of 3 months. Haemoglobin measurements were conducted at baseline and monthly, while serum iron, serum ferritin, transferritin, and TIBC were measured at baseline and after 3 months. The anaemia profile was similar between groups at baseline. Haemoglobin levels were significantly increased after 1 month in the high ferric sodium EDTA group compared to the control and low ferric sodium EDTA groups; however, after 2 and 3 months, haemoglobin levels were significantly higher in both ferric sodium EDTA groups compared to controls and baseline. Serum iron and serum ferritin significantly increased and free erythrocyte protoporphyrin (FEP), TIBC, and transferritin levels significantly decreased compared to baseline and compared to controls in both ferric sodium EDTA groups. With the exception of haemoglobin levels after 1 month, there were no significant differences in any measure of iron status between the ferric sodium EDTA groups. The results indicate that the addition of ferric sodium EDTA to the diet at levels providing 5 and 20 mg iron/day significantly improved iron status among anaemic children, with no reports of any significant adverse effects.

3.5.6.2 Ferric Sodium EDTA-Fortified Sugar

A double-blind controlled field study was conducted among low-income, semi-rural communities in Guatemala, whereby 2 lowland (T_1 and T_2) and 1 highland (T_3) communities were provided with sugar fortified with approximately 15 mg vitamin A/kg sugar and 124 mg iron/kg sugar (administered as 1 g ferric sodium EDTA/kg sugar) for a period of 32 months (Viteri *et al*., 1995). For control, another highland Guatemalan community received sugar that had been fortified only with vitamin A. The population of the T_1, T_2, T_3, and control communities were reported to be 1,144, 1,756, 1,645, and 1,095, respectively. The target sample size from each community was 318 subjects, with 160 randomly chosen children and adults having mean haematocrit values less than -1 standard deviation (SD) from that of the mean haematological norms for Central America, and 158 subjects having mean

haematocrit values of at least -1 SD from the norm. Excluded from analysis were anaemic subjects treated with $FeSO_4$ tablets, severely undernourished children, and pregnant women. The fortified sugar was supplied to local stores, where community members normally purchased their sugar. To ensure compliance and to check for possible infiltration of unfortified sugar in the experimental communities, samples of sugar were obtained at random periods of time from all of the households and analyzed. Overall household compliance figures were conservatively estimated to be 72, 75, 93, and 0% for the T_1, T_2, T_3, and control communities, respectively, which indicated a persistent infiltration of unfortified sugar in the lowland test communities and an absence of ferric sodium EDTA-fortified sugar in the control. Sugar consumption in all of the 4 communities studied ranged from 34.6 to 37.8 g/person/day (4.3 to 4.7 mg iron/day). The ferric sodium EDTA-fortified sugar was very stable, except for a slow progressive discolouration to a brownish hue after 6 months of storage in the most severe conditions. Overall, the addition of ferric sodium EDTA-fortified sugar in typical recipes was considered acceptable, with some instances in which the colour of the food appeared slightly darker.

Mean iron absorption estimates for different communities, and age and sex groups ranged from 0.95 to 3.06 mg iron/day, or 3.5 to 14.1% of total iron intake. There were no undesirable effects as a result of the ferric sodium EDTA fortification of sugar. Blood samples were obtained from a cohort of subjects belonging to the unfortified and fortified communities at 0, 8, 20, and 32 months of fortification. Measurements of iron nutrition indicators included serum haemoglobin, percent saturation of TIBC, FEP, serum ferritin, and iron stores. The effects of consuming ferric sodium EDTA-fortified sugar on different iron nutrition indicators are listed in Table 3.5.6.2-1. In general, baseline measures of iron status indicated that the lowland communities (T_1 and T_2) were iron-deficient in comparison to the highland (T_3) and control communities, with community T_2 being more deficient. Significant improvements in iron status from baseline were observed in the fortified communities T_1, T_2, and T_3 after 32 months. A general trend in increasing final mean haemoglobin concentrations (towards normal values) was reported for all of the fortified communities; however, significant increases from basal levels were observed only in males and females (1 to 8 years of age) of communities T_1 and control, and males (=18 years of age) in community T_2. In contrast, haemoglobin levels significantly decreased in females (=45 years of age) and males (=18 years of age) in community T_3. The percent saturation of TIBC, a biological indicator of iron transport, significantly increased from basal values in all of the fortified community groups and approached normal values (*i.e.*, control values) by the end of the 32-month fortification study. Basal FEP values were elevated in the 2 lowland communities (T_2 in particular), indicating a state of iron-deficient erythropoiesis in these communities. FEP values significantly decreased from basal values after 32 months of fortification in the T_1, T_2, and T_3 communities, with the exception of T_1 and T_2 females (18 to 44 years of age), T_2 males and females (aged 9 to 17 years) and T_1 and T_3 males (=18 years of age). Final serum ferritin concentrations significantly increased over basal values in all aged groups in all fortified communities (T_1, T_2, and T_3). Within the control community, 3/5 groups were reported to have significant differences between basal and final serum ferritin values. Of these, serum ferritin levels significantly decreased in males and females (aged 9 to 17 years)

and significantly increased in males (=18 years of age) and females (=45 years of age). Mean individual iron stores were reported to increase with age in all 4 of the communities studied, being highest in females (=45 years of age) and males (=18 years of age). With the exception of females (aged 18 to 44 years) in the T_1 community and females (=45 years of age) in the T_3 community, significant increases in iron stores were reported in the T_1, T_2, and T_3 communities after 32 months of fortification. There were no significant increases in the iron stores of those in the control community, with the exception of males =18 years of age.

Table 3.5.6.2-1 Reported Effects of Ferric Sodium EDTA-Fortified Sugar on Mean Basal and Final Values of Iron Nutrition Indicators by Community Based on Final Age and Sex Groups[1]

Sex and Age Groups	Lowland Communities				Highland Communities			
	T_1		T_2		T_3		C^2	
	Basal	Final	Basal	Final	Basal	Final	Basal	Final
Haemoglobin (g/L)								
M and F (1-8 y)	120[a]	130[e,3]	122[a]	122[e]	134[b]	136[f]	132[b]	139[f,3]
M and F (9-17 y)	134[a]	137[e]	123[a]	131[e]	144[b]	145[f]	141[b]	148[f]
F (18-44 y)	131[a,b]	132[e]	127[b]	132[e]	140[c]	144[f]	138[a,c]	141[f]
F (= 45 y)	133[a]	136[e]	123[b]	119[f]	146[c]	138[c,3]	141[a,c]	141[e]
M (= 18 y)	149[a]	144[e]	132[b]	144[e,3]	163[c]	155[f,3]	154[a]	155[f]
Saturation of total iron binding capacity (%)								
M and F (1-8 y)	20.3[a,b]	26.0[e,f,3]	17.0[a]	22.5[e,3]	22.2[b,c]	30.1[f,3]	24.5[c]	30.4[f,3]
M and F (9-17 y)	23.4[a]	28.4[e,3]	15.1[b]	21.8[f,3]	21.6[a]	32.1[e]	27.1[c]	29.4[e]
F (18-44 y)	19.1[a]	22.9[e]	18.9[a]	24.1[ef]	22.4[a]	31.4[g,3]	29.8[b]	30.3[f,g]
F (=45 y)	23.7[a,b]	31.0[e,3]	17.9[a]	25.4[e,3]	27.1[b]	30.2[e]	29.0[b]	30.3[e]
M (=18 y)	27.1[a]	28.7[e]	20.1[b]	29.6[e,3]	30.6[a]	36.6[f,3]	27.3[a]	33.6[e,f,3]
Free erythrocyte protoporphyrin (µg/L red blood cells)								
M and F (1-8 y)	1,520[a]	800[e,3]	1,190[a]	1,090[e,3]	770[b]	470[f,3]	560[b]	460[f]
M and F (9-17 y)	860[a]	620[e,3]	1,340[b]	910[f]	570[a]	430[e,3]	500[a]	460[e]
F (18-44 y)	1,190[a]	820[e]	1,190[a,b]	890[e]	860[b,c]	490[f,3]	580[c]	530[f]
F (=45 y)	1,160[a,b]	650[e,3]	1,460[b]	620[e,3]	850[a]	550[e,3]	800[a]	890[e]
M (=18 y)	780[a]	560[e,f]	1,110[b]	610[g,3]	460[c]	420[f]	660[a,b]	490[e,f,3]
Serum ferritin (µg/L)								
M and F (1-8 y)	8.3[a]	20.3[e,3]	5.9[a]	15.9[f,3]	16.5[b]	26.0[g,3]	15.4[b]	15.8[f]
M and F (9-17 y)	13.9[a]	22.1[e,3]	6.1[b]	18.7[f,3]	19.0[c]	25.4[g,3]	20.3[a]	15.0[h,3]
F (18-44 y)	11.9[a]	21.1[e,3]	7.4[b]	17.0[f,3]	15.3[c]	25.3[g,3]	19.9[a]	19.1[h]
F (= 45 y)	19.4[a]	44.7[e,3]	8.5[b]	31.5[f,3]	39.3[c]	46.9[g,3]	29.3[d]	30.6[h,3]
M (= 18 y)	22.4[a]	31.4[e,3]	7.5[b]	21.3[f,3]	29.9[c]	46.9[g,3]	21.8[d]	24.4[f,3]

Table 3.5.6.2-1 Reported Effects of Ferric Sodium EDTA-Fortified Sugar on Mean Basal and Final Values of Iron Nutrition Indicators by Community Based on Final Age and Sex Groups[1]

Sex and Age Groups	Lowland Communities				Highland Communities			
	T_1		T_2		T_3		C^2	
	Basal	Final	Basal	Final	Basal	Final	Basal	Final
Iron stores (mg)[4]								
M and F (1-8 y)	-26[a]	41[e,3]	-35[a]	9[e,3]	21[b]	79[f,3]	24[b]	31[e]
M and F (9-17 y)	30[a]	150[e,3]	-85[b]	42[f,3]	71[a,c]	171[e,3]	80[c]	81[f]
F (18-44 y)	8[a,b]	68[e]	-156[b]	67[e,3]	37[a]	182[e,3]	128[a]	122[e]
F (=45 y)	24[a]	206[e,f,3]	-91[a]	124[e,3]	262[b]	307[f]	215[b]	199[e,f]
M (=18 y)	168[a]	333[e,3]	-157[b]	169[e,3]	347[c]	490[f,3]	184[a]	277[e,3]

Viteri *et al*., 1995

[a, b, c, d, e, f, g, h] Means with the same superscripts do not differ from each other.

[1] Values with different superscript letters are significantly different from one another (between-community comparisons for a particular age and sex group), $P < 0.05$.

[2] Control community

[3] Significantly different from basal value within the same community group, $P < 0.05$ (paired *t* test).

[4] Negative iron stores indicate iron deficiency.

The more deficient groups (*i.e.*, communities T_1 and T_2) exhibited the fastest and the greatest positive responses to iron fortification than any of the other groups (*i.e.*, communities T_3 and control). These responses were reported to decrease with time, as iron deficiencies began to disappear and iron stores started to build up in the subjects. Using the criteria for estimating the prevalence of anaemia (corrected for altitude), as set by the WHO, the authors reported that the prevalence of mild to moderate anaemia declined significantly in the lowland communities from 27.3 to -13.9% in community T_1 and from 41.7 to -16.9% in community T_2. In contrast, only a slight decline in the prevalence of anaemia was reported for the highland communities; from 10.2 to 7.6% in community T_3 (non-significant change) and from 13.9 to 5.2% in the control community (significant change). The authors had no explanation for the improvement in iron nutrition observed within the control community. However, they concluded that sugar fortification with ferric sodium EDTA was very effective in improving iron nutrition in populations with a high prevalence of anaemia, and that iron stores tend to stabilize at certain levels depending on the age, sex and community, approaching new equilibrium conditions in iron status by the 32nd month of fortification; the latter being dictated by the extent of iron requirement and/or need.

3.5.6.3 Ferric Sodium EDTA-Fortified Curry Powder

A 2-year, double-blind controlled iron fortification trial was conducted in an iron-deficient South African population of Indian descent, using masala (curry powder) fortified with ferric sodium EDTA at a level of 10 mg/g masala (corresponding to 25 µmol iron/g masala) (Ballot *et al*., 1989a). Using the average consumption rate of masala of 5.5 g/person/day (Ballot *et al*., 1989b), these levels correspond to doses of approximately 55 mg ferric sodium EDTA/day and 7.3 mg iron/day. A total of 264 families (984 subjects) participated in the

study, and were divided into control (129 families) and fortified (135 families) groups. Serum ferritin and haemoglobin concentrations and percentage saturation of transferrin were evaluated annually from blood samples obtained from the subjects. Blood samples also were obtained at the end of the 2-year period from a random sample of 30 individuals per group to determine plasma zinc levels, which were reported not to be significantly different between groups. Measurements of iron status from baseline improved over the course of the 2-year study in both control and fortified groups, with the greatest improvement occurring over the first year of the study. Improvement in iron status continued over the second year in the fortified group only. Statistically significant increases in haemoglobin levels were noted only in the female fortified group during year 2, while serum ferritin levels were significantly increased from baseline in the male fortified group after 2 years of fortification. Mean body iron stores also progressively improved in males and females in the fortified group. When the male fortified group was divided into adults and those <18 years of age, only the younger group had significantly increased serum ferritin compared to baseline and calculated body iron stores compared to controls. There were no significant differences in calculated body iron stores between adults of the fortified group and the controls. Although the improvement in haemoglobin levels reported in the females was apparent after 1 year of fortification, it only reached statistical significance (compared to controls) at the end of the 2nd year of fortification. In contrast, both males and females of the fortified group exhibited significant improvements in serum ferritin and calculated body iron stores at the end of the 1st year of fortification (compared to controls), which was maintained after 2 years. Males of the fortified and unfortified groups were reported to show a trend in improvement of iron status, but the difference between groups was not significant. Females of the fortified group, who were reported to have the highest prevalence of iron-deficiency anaemia at the beginning of the study than any of the other groups, exhibited a 41% improvement in iron status (*i.e.*, from 22 to 4.9%) compared to the 26% improvement observed in the controls (*i.e.*, from 17.4 to 13.3%). Similarly, females in the control group, who were iron-deficient at the start of the fortification study, showed the greatest improvement in iron status, compared to their iron-repleted counterparts. No significant increase in body iron stores was observed between male drinkers and non-drinkers of the control group, but significantly higher body iron stores were reported for male drinkers of the fortified group compared to controls. However, the mean change in body iron stores between the control and fortified group was not significantly different.

3.6 Data on Sources Containing or Derived from Genetically Modified Organisms

Ferric sodium EDTA does not contain, nor is it derived from genetically modified organisms.

4.0 SUMMARY AND CONCLUSIONS

Ferric sodium EDTA consists of one molecule of ferric iron bound to a hexadentate chelator, EDTA, *via* 4 carboxylate and 2 tertiary amine groups (Heimbach *et al*., 2000). Ferric sodium EDTA is a free-flowing yellow-brown powder with the chemical composition FeNa-EDTA·$3H_2O$. It can be isolated *via* crystallization with a high level of chemical purity. For food fortification and dietary supplementation purposes, ferric sodium EDTA is formulated as Ferrazone® ferric sodium EDTA.

The absorption of iron from ferric sodium EDTA is regulated through the same physiological mechanisms as other forms of iron. Following oral administration, the iron from ferric sodium EDTA, which is separated from the iron EDTA complex in the lumen of the gut, joins the general non-haem iron pool that is finally incorporated into the circulating haemoglobin. Less than 1% of the intact ferric sodium EDTA chelate is absorbed in humans, which is, in turn, rapidly excreted by the kidneys. Following dissociation from ferric sodium EDTA, EDTA is promptly excreted in the faeces, while less than 5% is absorbed and excreted in the urine. The iron component of ferric sodium EDTA is subsequently handled systemically like any other source of iron; the safety and maximum tolerable intake of which has been reviewed and evaluated by a number of distinguished scientific committees such as JECFA (1970, 1974, 1983; WHO, 1973), the UK EVM (2003), the SCF (1993), the IOM (2001), and EFSA (2004).

While a tolerable upper intake level has yet to be established for iron within the E.U., other organisations have published their recommendations. JECFA, in 1983, established a provisional Maximum Tolerable Daily Intake of 0.8 mg/kg body weight (48 mg/person/day) (JECFA, 1983), the IOM set an Upper Limit of 45 mg/person/day for adults (IOM, 2001), and the EVM published a guidance value of 17 mg/person/day (UK EVM, 2003). These recommendations, although seen to vary somewhat, provide assurance that dietary supplementation with iron sources such as ferric sodium EDTA would pose no safety concerns at these established levels, especially since the intended level of use of ferric sodium EDTA in fortified foods will not exceed those levels anticipated through existing iron supplementation and food fortification programmes currently used within the E.U. Furthermore, EDTA poses no safety concerns since calcium disodium EDTA (E385) is currently permitted for use as a food additive in the E.U. under *Directive 95/2/EC of the European Parliament and the Council of 20 February 1995 on food additives other than colours and sweeteners* (European Parliament and The Council of the European Union, 1995).

Due to its high solubility at physiological pH, the iron from ferric sodium EDTA is highly bioavailable, despite the presence of inhibitors of iron absorption in foods. Numerous studies comparing the absorption of iron from different sources, such as ferric sodium EDTA, $FeSO_4$, $Fe_2(SO_4)_3$, $FeCl_3$, and ferrous fumarate, have indicated significantly higher absorption of iron from ferric sodium EDTA, particularly in subjects with low iron status. When added to inhibitory meals (*i.e*., those containing iron inhibitors), the iron from ferric

sodium EDTA is 2 or 3 times as well absorbed as that from $FeSO_4$; however, in foods of high bioavailability (*e.g.*, those containing ascorbic acid), iron absorption is similar between ferric sodium EDTA and $FeSO_4$. Other studies have demonstrated that iron absorption is precisely regulated by the body. The percentage of iron absorption from foods was noted to decrease by almost a factor of 2 when the level of iron as ferric sodium EDTA was increased from 5 to 15 mg.

Ferric sodium EDTA has a low acute toxicity, with an oral LD_{50} value of 10,000 mg/kg body weight in rats, corresponding to approximately 1,326 mg iron/kg body weight. In various *in vitro* mutagenicity assays conducted in *S. typhimurium, E. coli*, mouse lymphoma L5178Y cells, and mouse bone marrow cells, ferric sodium EDTA exhibited no significant mutagenic potential. Gavage treatment of female Sprague-Dawley rats with 200 mg/kg body weight/day of ferric sodium EDTA (approximately 26.5 mg iron/kg body weight/day) on GD 7 to 16 produced no toxicologically significant effects on reproductive or developmental parameters. Additionally, dietary administration of up to 400 mg/kg body weight/day of ferric sodium EDTA (approximately 53.0 mg iron/kg body weight/day) did not induce sperm cell malformation in groups of male Kunming mice.

No adverse effects were reported in rats provided with 250 mg/kg body weight/day of ferric sodium EDTA (approximately 33.2 mg iron/kg body weight/day) in the diet for a period of 90 days. However, on the basis of a 61-day feeding study in rats provided with up to 11.2 mg iron/kg body weight/day, JECFA (2000) concluded that administration of ferric sodium EDTA in the diet would not result in a greater uptake of iron once nutritional requirements for iron are met, and that the use of ferric sodium EDTA would be safe for use in food fortification programs that would provide 0.2 mg iron/kg body weight/day (approximately 12.0 mg iron/ person/day) from fortified foods.

The safety of ferric sodium EDTA is further supported by subchronic, reproductive and developmental, and carcinogenicity studies in rats, mice, and dogs of other sources of EDTA. As previously discussed, 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. No compound-related mortality, reproductive, or teratogenic effects were reported in rats orally dosed with, EDTA, disodium EDTA, trisodium EDTA, calcium disodium EDTA, and tetrasodium EDTA, respectively, on GD 7 to 14. Furthermore, no significant reproductive or developmental effects were observed in multiple generations of rats exposed to diets containing calcium disodium EDTA. Results of 2-year feeding studies in rats and mice indicate that disodium EDTA, trisodium EDTA, and calcium disodium EDTA do not possess carcinogenic activity. A NOEL of 250 mg/kg body weight/day was reported for calcium disodium EDTA using 1- and 2-year feeding studies in dogs and rats, respectively. Based on this data, JECFA (1966, 1974) assigned an ADI of 2.5 mg/kg body weight/day (approximately 150 mg/person/day) for EDTA as calcium disodium EDTA. In 2007, JECFA re-confirmed this ADI and also expressed it as equivalent to 1.9 mg/kg bw/day of EDTA (JECFA,2007). The lack of significant toxicity of EDTA compounds is consistent with a

history of safe use of calcium disodium EDTA and disodium EDTA as preservatives, processing aids, and colour stabilizers in many foods.

Ferric sodium EDTA has been used in numerous field trials in developing countries for the iron fortification of foods. 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 (0.07 to 0.25 mg iron/kg body weight/day). Remarkable improvements in iron status indicators were reported in the fortified groups compared to the controls, with no evidence of iron overload. 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. In addition, studies evaluating the nutritional safety of ferric sodium EDTA in both rats and humans have demonstrated that ferric sodium EDTA does not adversely affect the absorption of other nutrients, including zinc, copper, calcium, magnesium, and manganese.

Overall, the results of toxicological and clinical studies of ferric sodium EDTA and other sources of iron and EDTA support the safe intake of Ferrazone® ferric sodium EDTA by humans. On an absolute basis, the greatest mean and 95th percentile all-user intakes of iron from all typical example fortified food-uses of Ferrazone® ferric sodium EDTA in the E.U. were determined in male adults (4.93 mg/person/day or 0.06 mg/kg body weight/day) and female adults (13.28 mg/person/day or 0.20 mg/kg body weight/day), respectively, whereas the lowest all-user mean and 95th percentile intakes of iron were determined in children, at 2.21 and 4.77 mg/person/day (0.15 and 0.33 mg/kg body weight/day), respectively. These consumption estimates are more than 2-fold lower than the background intakes of iron from food, water, and supplements in the U.K., which have been estimated to be 44 mg/person/ day (UK EVM, 2003). It is important to note that iron levels used in current fortification programs are well below the tolerable intakes for iron of 48 mg/person/day (0.8 mg/kg body weight/day) as set by JECFA (1983), or 45 mg/person/day (0.75 mg/kg body weight/day) of iron from all sources as presented by the IOM (2001). Hence, dietary levels of ferric sodium EDTA that provide less than 45 mg/person/day of iron would be considered safe for humans. Moreover, the highest mean and 95th percentile all-user intakes of EDTA, on a body weight basis, of 0.90 and 1.85 mg/kg body weight/day by children from all typical Example fortified food-uses of ferric sodium EDTA and all current food-uses of calcium disodium EDTA in the E.U. are less than the ADI of 2.5 mg/kg body weight/day for calcium disodium EDTA established and re-confirmed by JECFA as equivalent to 1.9 mg/kg bw/day of EDTA (1974, 2007). Therefore, the levels of exposure resulting from the conditions of use of ferric sodium EDTA comply with E.U. regulations and scientific opinions.

The scientific evidence presented above indicates that Ferrazone® ferric sodium EDTA would not produce adverse effects on human health under the intended conditions of use in foods as described herein. Furthermore, on the basis of the available bioavailability, metabolism, and toxicity data, and dietary supplementation and fortification studies in humans, the use of ferric sodium EDTA, meeting the specifications prepared herein, as a direct replacement for permitted iron forms in all PARNUTS categories other than baby

foods and infant formula *(Council Directive 89/398/EEC)* (Council of the European Communities, 1989), food supplements *(Directive 2002/46/EC)* (European Parliament and The Council of the European Union, 2002*)*, and fortified foods would not present a safety concern.

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