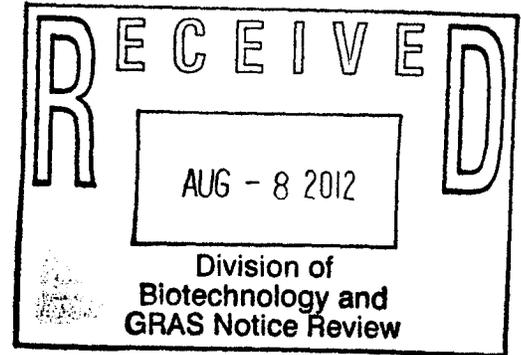


ORIGINAL SUBMISSION



**Eisai Food & Chemical Co., Ltd.**  
2-13-10 Nihonbashi, Chuo-ku, Tokyo, 103-0027 Japan



July 30, 2012

Dr. Mary Ditto  
Office of Food Additive Safety (HFS-200)  
Center for Food Safety and Applied Nutrition  
Food and Drug Administration  
5100 Paint Branch Parkway  
College Park, MD 20740-3835

Dear Dr. Ditto:

**Re: GRAS Notification for Sodium Ferrous Citrate**

In accordance with proposed 21 CFR §170.36 [Notice of a claim for exemption based on a Generally Recognized as Safe (GRAS) determination] published in the *Federal Register* [62 FR 18938 (17 April 1997)], I am submitting in triplicate, as the notifier [Eisai Food & Chemical Co., Ltd., 5th Floor Nihonbashi Sunrise Bldg., 2-13-10 Nihonbashi Chuo-ku Tokyo, 103-0027, Japan], a Notice of the determination, on the basis of scientific procedures, that sodium ferrous citrate, produced by Eisai Food & Chemical Co., Ltd., as defined in the enclosed documents, is GRAS under specific conditions of use as a food ingredient, and therefore, is exempt from the premarket approval requirements of the *Federal, Food, Drug and Cosmetic Act*. Information setting forth the basis for the GRAS determination, which includes detailed information on the notified substance, a summary of the basis for the GRAS determination, as well as a consensus opinion of an independent panel of experts in support of the safety of sodium ferrous citrate under the intended conditions of use, also are enclosed for review by the agency. Additionally, included on 1 CD-ROM, I am submitting English translations of the Japanese publications corroborating the GRAS status of sodium ferrous citrate, as requested by the Agency at the pre-notification meeting dated April 17<sup>th</sup>, 2012.

Should you have any questions or concerns regarding this GRAS Notice, please do not hesitate to contact me at any point during the review process so that we may provide a response in a timely manner.

Sincerely,

(b) (6)

Minoru Tanaka  
President  
Eisai Food & Chemical Co., Ltd.

000002



**Eisai Food & Chemical Co., Ltd.**

2-13-10 Nihonbashi, Chuo-ku, Tokyo, 103-0027 Japan

July 30, 2012

Dr. Mary Ditto  
Office of Food Additive Safety (HFS-200)  
Center for Food Safety and Applied Nutrition  
Food and Drug Administration  
5100 Paint Branch Parkway  
College Park, MD 20740-3835

Dear Dr. Ditto:

**Re: GRAS Notification for Sodium Ferrous Citrate**

Please be advised that Intertek Cantox is representing Eisai Food & Chemical Co., Ltd. and is fully authorized to act on our behalf with respect to the Generally Recognized as Safe (GRAS) notification for sodium ferrous citrate submitted on July 30, 2012.

Sincerely,

(b) (6)

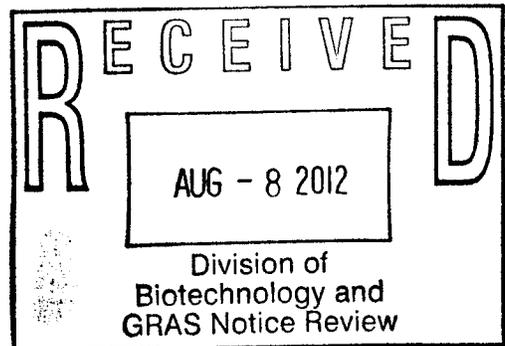
A large rectangular area of the document is redacted with a solid grey fill, obscuring the signature and name of the sender.

Minoru Tanaka  
President  
Eisai Food & Chemical Co., Ltd.

cc: Dr. Ashley Roberts, Intertek Cantox

000003

## GRAS Exemption Claim for Sodium Ferrous Citrate



**Submitted for:** Office of Food Additive Safety (HFS-200)  
Center for Food Safety and Applied  
Nutrition (CFSAN)  
Food and Drug Administration  
5100 Paint Branch Parkway  
College Park, MD 20740-383

**Submitted by:** Eisai Food & Chemical Co., Ltd.  
5th Floor Nihonbashi Sunrise Bldg.  
2-13-10 Nihonbashi Chuo-ku Tokyo  
103-0027 Japan

July 30, 2012

# GRAS Exemption Claim for Sodium Ferrous Citrate

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## I GRAS EXEMPTION CLAIM

### A. Claim of Exemption From the Requirement for Premarket Approval Pursuant to Proposed 21 CFR §170.36(c)(1) [62 FR 18938 (17 April 1997)]

Sodium ferrous citrate has been determined to be Generally Recognized as Safe (GRAS), consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This determination is based on scientific procedures as described in the following sections, under the conditions of its intended use in food, among experts qualified by scientific training and expertise. Therefore, the use of sodium ferrous citrate in food as described below is exempt from the requirement of premarket approval.

Signed,

(b) (6)

\_\_\_\_\_  
Minoru Tanaka  
Eisai Food & Chemical Co., Ltd.  
5<sup>th</sup> Floor Nihonbashi Sunrise Bldg.  
2-13-10 Nihonbashi Chuo-ku Tokyo  
103-0027 Japan

Date

July 30, 2012

### B. Name and Address of Notifier

Minoru Tanaka  
Eisai Food & Chemical Co., Ltd.  
5<sup>th</sup> Floor Nihonbashi Sunrise Bldg.  
2-13-10 Nihonbashi Chuo-ku Tokyo  
103-0027 Japan  
Telephone: +81-3-3548-3560  
Facsimile: +81-3-3273-2084  
Email: [m-tanaka@eisai-fc.co.jp](mailto:m-tanaka@eisai-fc.co.jp)

### C. Common Name of the Notified Substance

Sodium ferrous citrate

#### **D. Conditions of Intended Use in Food**

Eisai Food & Chemical Co., Ltd. (Eisai) intends to market sodium ferrous citrate (SFC) as a nutritive ingredient for use in foods. SFC is proposed as a direct replacement for other iron sources in existing categories of fortified foods in the U.S. Iron compounds are incorporated into foods in order to help meet dietary recommendations. Several iron compounds are affirmed as GRAS as direct food substances by the U.S. Food and Drug Administration (FDA). Consistent with the FDA's Fortification Policy and with 21 CFR 184.1(b)(1) (U.S. FDA, 2011), SFC is intended to be used in the same food categories as other iron salts at levels based on current Good Manufacturing Practice (cGMP). Based on the results of studies demonstrating the efficient absorption of SFC in comparison to other iron salts, such as ferrous sulfate (see Section IV.C), it is expected that the use levels of SFC required to achieve optimal blood concentrations of iron could be lower than the recommended intakes of other iron salts. As such, the consumption of SFC is not expected to significantly affect current iron intakes.

#### **E. Basis for the GRAS Determination**

Pursuant to 21 CFR § 170.30, SFC has been determined to be GRAS on the basis of scientific procedures. This determination is based on the views of experts who are qualified by scientific training and experience to evaluate the safety of sodium ferrous citrate as a component of food. The safety of SFC is supported by a number of published studies on the individual components, as well as the parent compound, as discussed herein. Furthermore, information to support the general recognition of the safe use of SFC is based on the opinions of internationally acclaimed scientific and regulatory agencies (JECFA, IOM and FDA) on the safety of sodium, citrate, and iron, the expected metabolic fate of SFC and its individual components, and preclinical and human studies conducted with SFC corroborating the authoritative reviews.

This determination is further supported by an expert panel evaluation of the health aspects of SFC. [See Appendix A – **Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of Sodium Ferrous Citrate for Use as a Food Ingredient**].

At the request of Eisai, an Expert Panel (“the Expert Panel”) of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, was specially convened on October 28, 2011 to conduct a critical and comprehensive evaluation of the available pertinent data and information, and to determine whether the intended uses of SFC as a nutritive ingredient, specifically a dietary source of iron, are safe and suitable and would be GRAS based on scientific procedures. The Panel consisted of the following qualified scientific experts: John Doull, Ph.D., M.D. (University of Kansas Medical Center), Robert J. Nicolosi, Ph.D. (University of Massachusetts Lowell), and Stephen L. Taylor, Ph.D. (University of Nebraska).

## Sodium Ferrous Citrate GRAS Exemption Claim

The Expert Panel, convened on behalf of Eisai, independently and collectively, critically evaluated the data and information summarized herein and concluded that the intended uses in traditional foods described herein for SFC, meeting appropriate food-grade specifications and manufactured according to cGMP, are safe and suitable and GRAS based on scientific procedures. It also is the Expert Panel's opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion.

SFC is GRAS based on scientific procedures for its intended use as a nutritive ingredient, specifically a dietary source of iron; therefore, it is excluded from the definition of a food additive and may be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21 of the CFR.

### F. Availability of Information

The data and information that serve as the basis for this GRAS Notification will be sent to the FDA upon request, or will be available for review and copying at reasonable times at the offices of:

Minoru Tanaka  
Eisai Food & Chemical Co., Ltd.  
5<sup>th</sup> Floor Nihonbashi Sunrise Bldg.  
2-13-10 Nihonbashi Chuo-ku Tokyo  
103-0027 Japan

Should the FDA have any questions or additional information requests regarding this notification, Eisai will supply these data and information.

## II. DETAILED INFORMATION ABOUT THE IDENTITY OF THE SUBSTANCE

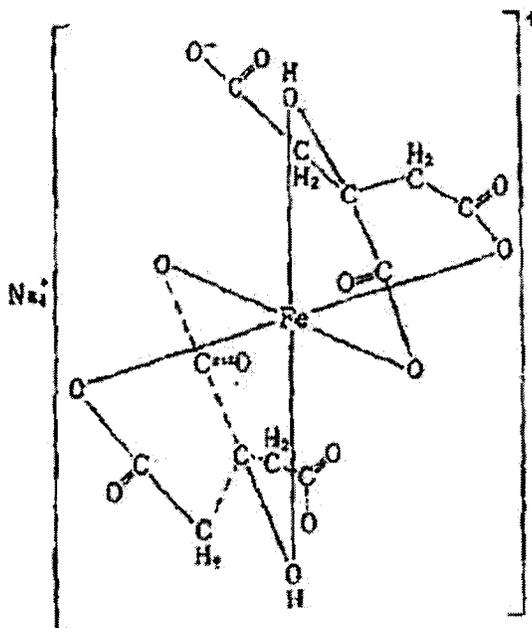
### A. Identity

SFC is an odorless, green-white crystalline powder with a weak iron taste and is readily soluble in acidic solvents, practically insoluble in water, and insoluble in basic solvents (Ishino *et al.*, 1988).

<b>Common or Usual Name:</b>	Sodium Ferrous Citrate
<b>Chemical Name:</b>	Tetrasodium bicitrate iron (II)
<b>Chemical Abstracts Service (CAS) Number:</b>	50717-86-7
<b>Empirical Formula and Formula Weight:</b>	$\text{Na}_4\text{FeC}_{12}\text{H}_{10}\text{O}_{14}$
<b>Molecular weight:</b>	526.01

## Sodium Ferrous Citrate GRAS Exemption Claim

### Structural Formula:



### B. Composition

Details regarding the composition of SFC, including qualitative and quantitative assays are described in Ishino *et al.* (1988). Tests for the presence of ferrous salts, citric acid, sodium salts, and complex salts, performed in accordance with the 5<sup>th</sup> Edition of the Japanese Standards for Food Additives (MHLW, 1987), confirmed the presence of ferrous salts, citric acid, and sodium salts. The total iron and citrate content of SFC was determined by iodometric and neutralization titration methods, respectively, as described by Ishino *et al.* (1988). The content of ferric ions also was determined. As presented in Table II.B-1, the average content of total iron, citrate, and ferric ions in SFC is 10.4, 72.0, and 0.05%, respectively, with a molar ratio of sodium, citrate, and iron(II) being 4:2:1. Based on the content of total iron, citrate, and ferric ions in SFC, the sodium content was calculated to be approximately 17.6%.

**Table II.B-1 Analytical Data Relating to the Composition of Five Batches of Sodium Ferrous Citrate**

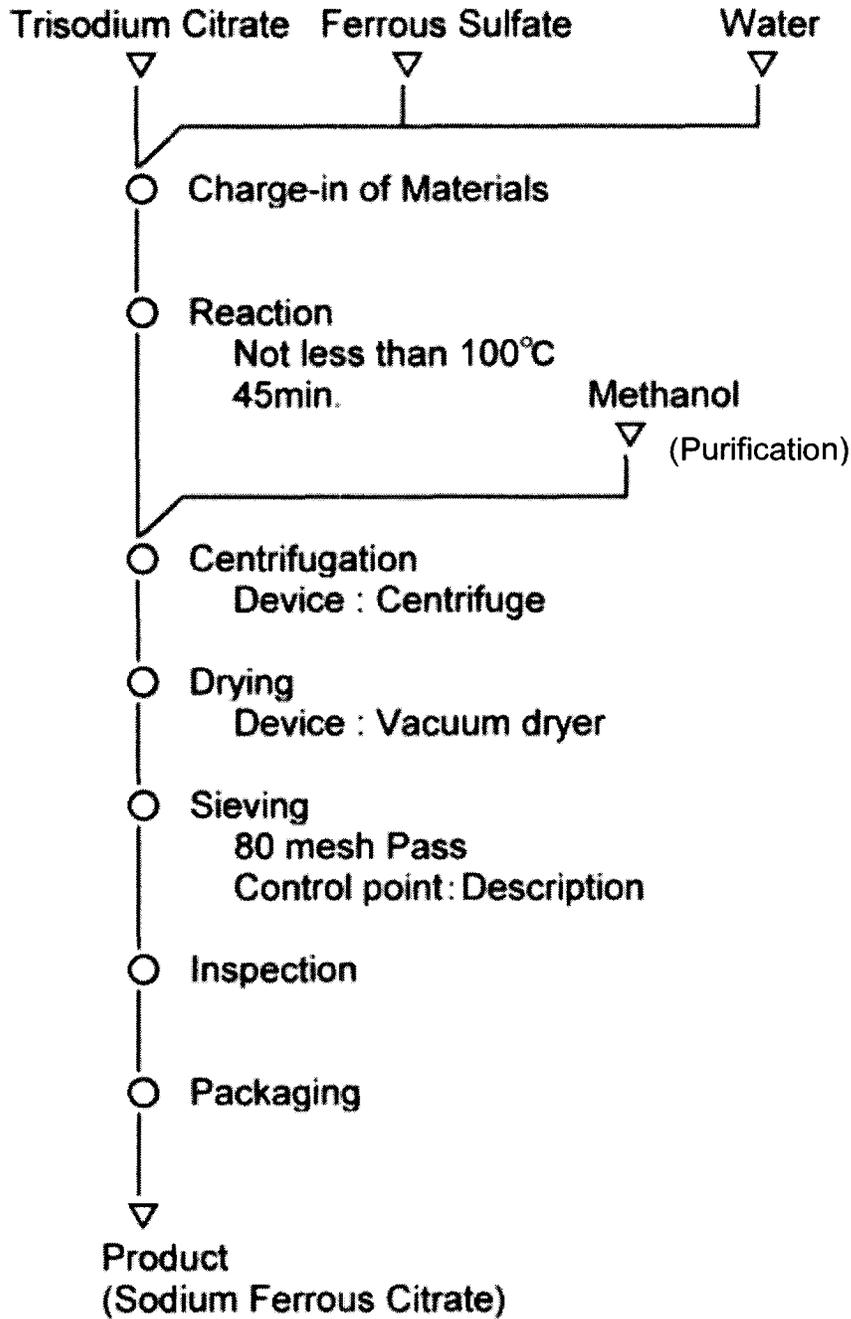
Parameter	Batch Number				
	60721	60722	60902	60908	71130
Total Iron (%)	10.4	10.5	10.5	10.4	10.3
Ferric Iron (%)	0.07	0.07	0.08	0.03	0.02
Citrate (%)	72.3	72.3	71.5	72.1	71.7

Adapted from Ishino *et al.* (1988)

**C. Method of Manufacture**

A schematic diagram of the general manufacturing process employed to produce SFC is illustrated in Figure II.C-1. SFC is produced in accordance with cGMP *via* a chemical reaction between sodium citrate and ferrous sulfate. Trisodium citrate, ferrous sulfate, and water are mixed and allowed to react for 45 minutes at 100°C. Methanol is added to the reaction products to precipitate the salt, which is subsequently dried and sieved to yield a final product designated as Sanferol (trade name). All raw materials used in the production of SFC meet “food-grade” specifications as well as the Japanese Standards for Food Additives. Sodium citrate is affirmed as GRAS for use in food with no limitations other than cGMP (21 CFR §184.1751). Ferrous sulfate also is affirmed as GRAS for use as a nutritional supplement with no limitations other than cGMP (21 CFR §184.1315).

Figure II.C-1 Schematic Overview of the Manufacturing Process for Sodium Ferrous Citrate



**D Specifications for Food Grade Material****D.1 Chemical and Microbiological Specifications for Food Grade Material**

The chemical specifications for SFC are presented in Table II.D.1-1 and the microbiological specifications for SFC are presented in Table II.D.1-2. All analytical methods corresponding to the chemical specifications follow established standards as described by the Japanese Standards of Food Additives. Eisai has developed methods for microbiological analysis, which are based on the Microbiological Test described in the General Tests of the Japanese Pharmacopoeia. Analyses of 3 representative, non-consecutive lots demonstrated compliance with final product chemical and microbiological specifications (please see Appendix B).

Specification Parameter	Specification	Method
Identification	Meets requirements	MHLW (2009). Japanese Standards of Food Additives, 8 <sup>th</sup> Edition, pp. 362-363.
Assay	10 to 11% Fe (mw = 55.85)	MHLW (2009). Japanese Standards of Food Additives, 8 <sup>th</sup> Edition, pp. 362 and 363.
Sulfate	NMT 0.48%	MHLW (2009). Japanese Standards of Food Additives, 8 <sup>th</sup> Edition, pp. 363
Ferric salt	Meets requirements	MHLW (2009). Japanese Standards of Food Additives, 8 <sup>th</sup> Edition, pp. 363
Heavy metals	NMT 20 ppm	MHLW (2009). Japanese Standards of Food Additives, 8 <sup>th</sup> Edition, pp. 363
Lead	NMT 1 ppm	FCC p1083 (III) (FCC VII General Tests and Assays "Lead Limit Test")
Arsenic	NMT 4.0 ppm	MHLW (2009). Japanese Standards of Food Additives, 8 <sup>th</sup> Edition, pp. 363
Tartrate	Meets requirements	MHLW (2009). Japanese Standards of Food Additives, 8 <sup>th</sup> Edition, pp. 363
Foreign Matter	NMT 3	

Fe, iron; mw, molecular weight; NMT, not more than

Specification Parameters	Specification	Method
Total viable count		
Bacteria	NMT 100 CFU/g	Eisai Food & Chemical Co. Ltd.
Fungi	NMT 100 CFU/g	Eisai Food & Chemical Co. Ltd.
<i>Escherichia coli</i>	Negative	Eisai Food & Chemical Co. Ltd.
<i>Pseudomonas aeruginosa</i>	Negative	Eisai Food & Chemical Co. Ltd.
<i>Staphylococcus aureus</i>	Negative	Eisai Food & Chemical Co. Ltd.

CFU, colony forming units; NMT, not more than

## D.2 Additional Analyses

As described in Section II.C, methanol is used as a processing aid in the production of SFC. Although it is expected that the methanol is removed during vacuum drying, Eisai has analyzed 3 non-consecutive batches of SFC for the presence of methanol residues. The results demonstrated compliance with Eisai's specifications of not more than 50 µg/g of methanol.

## D.3 Stability

Ishino *et al.* (1988) describes the stability of SFC under various conditions. A summary of the results of varying stability conditions are provided below in Table II.D.3-1. Further details of the individual stability tests are provided in Sections II.D.3.1 to II.D.3.6, where available. The authors concluded that the color changes observed in the SFC solutions, depending on pH values, were due to the formation of chelate compounds at neutral pH and the dissociation of SFC under acidic conditions into sodium, iron, and citrate; SFC was satisfactorily stable under normal conditions.

Parameter	Storage Conditions	Results
Stability against heat under open conditions	Stored at 45°C for 3 months in a brown glass bottle	- Stable
Stability against humidity	Stored at 40°C and 75% RH for 1 month	- Change in color (from greenish white to yellow-greenish white) - Proportion of ferric iron to total iron increased (0.24%) - Loss on drying increased (0.5%)
Stability against light	Stored in the presence of sunlight for 2 weeks in a quartz tube	- Slight change in appearance - No ferric iron observed
Stability in aqueous solutions	Stored in a 1% aqueous solution at various pH values, stored at 37 or 60°C for 7 days in a brown glass bottle	- SFC dissolved in solution at all pH levels (1.1 to 8.1), color of solution varied with pH - No change in pH, HPLC, or citric acid measurements - Rate of ferric oxide formation increased with increasing pH (little oxidation at acidic pH, greater oxidation at weak acidic, neutral and basic pH)
	Stored in a 1% aqueous solution at various pH values stored at 37°C for 10 hours in a brown volumetric flask	
Stability in tablets	PTP product containing SFC stored at 40 or 45°C and 75% RH for 3 months under fluorescent light (1,000 lux)	- No change
	PTP product containing SFC stored at room temperature for 3.5 years	

**Sodium Ferrous Citrate GRAS Exemption Claim**

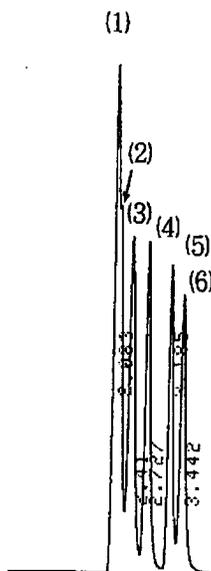
<b>Table II.D.3-1 Stability of Sodium Ferrous Citrate in Varying Storage Conditions</b>		
<b>Parameter</b>	<b>Storage Conditions</b>	<b>Results</b>
Stability of granules	Granule product of SFC stored at 40 or 45°C and 75% RH for 3 months	- No change
	Granule product of SFC stored at room temperature for 3 years	
	Granule product of SFC stored under open stress test conditions at 40°C and 75% RH for 1 week	- Change in color (from greenish white to green-yellowish white)
	Granule product of SFC stored under fluorescent light (1,000 lux) for 1 month.	- Slight increase in disintegration time

HPLC, high performance liquid chromatography; PTP, press through packaged; RH, relative humidity; SFC, sodium ferrous citrate

**D.3.1 HPLC Analysis of Sodium Ferrous Citrate Degradation Products**

As described by Ishino *et al.* (1988), high performance liquid chromatography (HPLC) was used to isolate and detect citric acid, aconitic acid, and other general organic acids (expected degradation products of citric acid), and the results are provided below in Figure II.D.3.1-1.

**Figure II.D.3.1-1 HPLC Chromatogram of SFC Degradation Products**



Citric acid (1), Tartaric acid (2), Aconitic acid(3), Succinic acid(4), Formic acid(5), Acetic acid(6)

**D.3.2 Stability of Sodium Ferrous Citrate against Heat**

The results of the heat stability test involving storage of SFC in open conditions at 45°C for 1 month in a brown glass bottle are presented below in Table II.D.3.2-1.

## Sodium Ferrous Citrate GRAS Exemption Claim

**Table II.D.3.2-1 Stability of Sodium Ferrous Citrate against Heat When Stored at 45°C for 1 Month**

Component	Initial	1 Month
Ferric salt	0.07%	0.06%
Iron content	10.4%	10.5%
Citrate content	72.4%	71.8%

### *D.3.3 Stability of Sodium Ferrous Citrate against Humidity*

The results of the humidity stability test involving storage of SFC at 45°C and 75% relative humidity for 1 month are presented below in Table II.D.3.3-1.

**Table II.D.3.3-1 Stability of Sodium Ferrous Citrate against Humidity When Stored at 45°C and 75% Relative Humidity for 1 Month**

Component	Initial	1 Month
Loss on drying	0.03%	0.51%
Ferric salt	0.02%	0.24%

### *D.3.4 Stability of Sodium Ferrous Citrate against Light*

The results of the photostability test involving storage of SFC in direct daylight for 2 weeks are presented below in Table II.D.3.4-1.

**Table II.D.3.4-1 Stability of Sodium Ferrous Citrate against Light When Stored in Direct Daylight for 2 Weeks**

Component	Initial	2 Weeks
Ferric salt	0.02%	0.03%

### *D.3.5 Stability of Sodium Ferrous Citrate in Aqueous Solution*

SFC in a 1% aqueous solution at various pH values, stored at 37 or 60°C for 7 days in a brown glass bottle was demonstrated to be stable, with no changes detected in the pH or citrate content of the solution (see Tables II.D.3.5-1 and II.D.3.5-2, respectively).

Sodium Ferrous Citrate GRAS Exemption Claim

Solution	Temperature	Day			
		Initial	1	3	7
0.2N HCl	37°C	1.1	1.1	1.1	1.0
	60°C		1.1	1.1	1.0
0.1N HCl	37°C	2.1	2.1	2.0	2.0
	60°C		2.1	2.0	2.0
1 <sup>st</sup> fluid (JP11)	37°C	3.4	3.3	3.3	3.3
	60°C		3.3	3.3	3.3
0.02N HCl	37°C	5.1	5.0	5.0	5.0
	60°C		5.0	5.1	5.1
2 <sup>nd</sup> fluid (JP11)	37°C	6.6	6.6	6.6	6.6
	60°C		6.6	6.6	6.6
pH 8 solution	37°C	8.1	8.1	8.1	8.1
	60°C		8.1	8.1	8.1
Water	37°C	6.7	6.6	6.6	6.6
	60°C		6.6	6.6	6.6

Solution	Temperature	Day			
		Initial (%)	1 (%)	3 (%)	7 (%)
0.2N HCl	37°C	100.0	98.6	99.3	99.4
	60°C		99.3	100.0	100.4
0.1N HCl	37°C	100.0	97.1	97.6	98.1
	60°C		98.8	98.3	99.2
1 <sup>st</sup> fluid (JP11)	37°C	100.0	97.1	97.6	98.1
	60°C		97.6	98.4	101.9
0.02N HCl	37°C	100.0	99.9	98.4	101.4
	60°C		99.4	100.0	98.4
2 <sup>nd</sup> fluid (JP11)	37°C	100.0	98.7	97.6	98.3
	60°C		98.7	98.5	99.2
pH 8 solution	37°C	100.0	101.3	100.8	100.8
	60°C		100.9	102.8	100.0
Water	37°C	100.0	98.6	97.9	98.8
	60°C		98.3	98.3	99.2

D.3.6 Long-Term Stability

In addition to the analyses conducted by Ishino *et al.* (1988), the long-term stability of SFC when stored for up to 3 years under ambient conditions has been evaluated by Eisai. As demonstrated in Table II.D.3.6-1, all parameters analyzed remained within the established tolerance limits after 3 years of storage. After 3 years of storage, the ferric salt content in Lot 6901 increased to 0.278%, and the sample appeared slightly brown because this sample was considered to be heat-sealed inadequately, however, the result for ferric salt is still below the established tolerance limit. Therefore, the shelf-life of SFC was confirmed to be 3 years when stored under ambient conditions.

Parameter	Tolerance limit (%)	Manufacturing Lot	Initial value (%)	Year 1 (%)	Year 2 (%)	Year 3 (%)
Ferric Salt	≤0.28	5802 (05052601)	0.06	0.043	0.073	0.00
		6901 (06062606)	0.01	NE	0.00	0.278
		76C59S (07060201)	0.01	0.02	0.093	0.096
Assay	10.0 to 11.0 as Fe	5802 (05052601)	10.3	10.31	10.15	10.35
		6901 (06062606)	10.4	NE	10.29	10.32
		76C59S (07060201)	10.4	10.25	10.36	10.40
Loss on Drying	≤0.1	5802 (05052601)	0.05	0.03	0.025	0.00
		6901 (06062606)	0.02	NE	0.00	0.00
		76C59S (07060201)	0.0	0.005	0.06	0.02

NE, not evaluated

**III. SELF-LIMITING LEVELS OF USE**

At levels significantly higher than those proposed, the use of SFC will result in unfavorable color, odor, and taste of the food. As a result, the food would become unpalatable thus the use of SFC is self limited by these factors.

**IV. BASIS FOR GRAS DETERMINATION**

**A. Introduction**

The determination that SFC is GRAS is on the basis of scientific procedures and the information supporting the general recognition of the safe use of SFC includes:

- Data pertaining to the identity, intended use, and estimated intake of SFC,
- The expected metabolic fate of SFC and its individual components

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- Authoritative reviews on the safety of sodium, citrate, and iron, and
- Preclinical and human studies corroborating the safety of SFC.

Moreover, these data were reviewed by a panel of experts, qualified by scientific training and experience to evaluate the safety of ingredients as components of food, who concluded that the intended uses of SFC are safe and suitable and would be GRAS based on scientific procedures [see Appendix A, **Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of Sodium Ferrous Citrate for Use as a Food Ingredient**].

### **B. Intended Use and Estimated Dietary Consumption of Sodium Ferrous Citrate in Food**

#### **B.1 Background Intake**

Iron compounds are incorporated into foods in order to help meet recommended dietary intakes at levels intended to supplement rather than replace iron taken in the normal diet. Several iron compounds are affirmed as GRAS as direct food substances by the FDA including ferric ammonium citrate, ferric chloride, ferric citrate, ferric phosphate, ferric pyrophosphate, ferric sulfate, ferrous ascorbate, ferrous carbonate, ferrous citrate, ferrous fumarate, ferrous gluconate, ferrous lactate, and ferrous sulfate with no limitation other than cGMP (21 CFR §184) (U.S. FDA, 2011). Moreover, sodium iron ethylenediaminetetraacetic acid (EDTA) (GRAS Notice No. GRN 000152 and GRN 000178) and ferrous ammonium phosphate (GRAS Notice No. GRN 000271) are considered GRAS for use as dietary iron sources for fortification purposes in selected foods, (U.S. FDA, 2004, 2006, and 2009 respectively). The FDA has set the reference daily intake (RDI), also referred to as the daily value (DV), of iron at 18 mg/person/day (21CFR §101.9) (U.S. FDA, 2011) where iron fortification salts are typically added as a percentage of the RDI in order to supplement iron intake from the normal diet.

The Institute of Medicine (IOM) in 2001 reviewed the available literature on iron to establish recommended daily allowances (RDAs) and tolerable upper intake limits (ULs) for iron for the different U.S. population groups (IOM, 2001). The RDAs and ULs for children ages 1 to 8 years and males and females ages 9 to >70 years, are summarized in Table IV.B.1-1. Furthermore, the IOM estimated the mean and 90<sup>th</sup> percentile (heavy consumer) daily intake of iron from all foods and supplements based on the data collected from the Third National Health and Nutrition Examination Survey (NHANES III; 1988-1994). These intake estimates also are presented in Table IV.B.1-1.

Life Stage Group		RDA for Iron (mg/person/day)	UL for Iron (mg/person/day)	Estimated Intake of Iron (mg/person/day)	
				Mean (mg/person/day)	90 <sup>th</sup> percentile (mg/person/day)
Children	1 to 3 years	7	40	10.36	17.60
	4 to 8 years	10	40	14.68	21.27
Males	9 to 13 years	8	40	18.05	25.70
	14 to 18 years	11	45	20.88	32.68
	19 to 30 years	8	45	20.87	31.84
	31 to 50 years			21.09	33.48
	51 to 70 years			20.64	34.30
	>70 years			20.95	34.50
Females	9 to 13 years	8	40	14.63	21.84
	14 to 18 years	15	45	13.24	19.61
	19 to 30 years	18	45	16.76	29.10
	31 to 50 years			17.11	31.01
	51 to 70 years	8	45	16.83	30.46
	>70 years			19.01	32.03
Pregnancy	14 to 50 years	27	45	48.97	88.84
Lactation	14 to 18 years	10	45	58.51	112.00
	19 to 50 years	9	45		

RDA, recommended daily allowance; UL, upper tolerable limit

## **B.2 Intended Use of Sodium Ferrous Citrate and Estimated Intake from Intended Uses**

Eisai intends to market SFC as a nutritive ingredient for use in foods. SFC is proposed for use as a direct replacement for other iron sources in existing categories of fortified foods for other iron salts in the U.S., at levels based on cGMP. Due to studies demonstrating the efficient absorption of SFC in comparison to other iron salts, such as ferrous sulfate (see Section IV.C.3), it is expected that the use levels of SFC required to achieve optimal blood concentrations of iron could be lower than the recommended intakes of other iron salts.

As SFC is intended to be used as a direct replacement of other iron fortification salts at similar or lower levels in food, the consumption of SFC is not expected to significantly affect current iron intakes. Therefore, an in-depth intake assessment was not conducted; rather, the intakes of iron from sodium ferrous citrate were assumed to be similar to those estimated by IOM for all foods and supplements. This substitution method was deemed to be appropriately conservative as it assumes that SFC would: 1) replace all iron currently available, including both naturally occurring, as well as supplemental; 2) be used in all food categories in which iron is present; and 3) be used at similar levels as other iron fortification salts. As presented in Table IV.B.1-1

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above, the mean and 90<sup>th</sup> percentile intakes of iron for all age groups, with the exception of pregnant and breast feeding women between the ages of 14 and 50 years, are more than 2.1 and 1.3 times lower, respectively, than the established upper tolerable limit (UL) for iron.

SFC is composed of sodium (17.6%), citric acid (72%), and iron (10.4%), as previously discussed in Section II.B. Accordingly, SFC also provides sodium and citric acid/citrates as dietary sources based on the absorption mechanism of SFC (see Section IV.C). Citrate is rapidly absorbed and utilized in oxidative metabolism (citric acid cycle); therefore, the potential intake of citrate/citric acid from SFC is expected to be of limited safety concern (see Section IV.C.2).

Due to some recent concerns regarding increasing intakes of salt (sodium) within the U.S. population (IOM, 2010), the potential intakes of sodium from SFC were calculated based on the sodium content of SFC (17.6%) and the estimated mean and 90<sup>th</sup> percentile dietary iron intakes from all foods and supplements presented in Table IV.B.1-1. Assuming that SFC would directly replace all sources of iron (including naturally occurring iron) from food and supplements at similar use levels, the mean and 90<sup>th</sup> percentile daily sodium intakes were calculated (see Table IV.B.2-1). Additionally, the current estimated mean and 90<sup>th</sup> percentile daily intake of sodium from foods as reported by the IOM is presented for comparative purposes. The mean calculated intake of sodium from SFC for all age groups, including pregnant and lactating women, is 31.04 mg/person/day, whereas the calculated 90<sup>th</sup> percentile intake of sodium for all age groups, including pregnant and lactating women is 50.99 mg/person/day. The calculated mean and 90<sup>th</sup> percentile sodium intakes from SFC are more than 100 times lower than the background mean and 90<sup>th</sup> percentile dietary sodium intakes estimated by the IOM. Therefore, the consumption of dietary sodium from the use of SFC is not expected to significantly increase the daily sodium consumption levels. Furthermore, the actual intakes of iron and sodium are expected to be much lower than those presented in Tables IV.B.1-1 and IV.B.2-1, respectively, because of the assumptions used when estimating the intakes of iron, as described above.

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Life Stage Group (maximum value/life stage group category)		Estimated Intake of Iron (mg/person/day) from Food and Supplements <sup>a</sup>		Estimated Intake of Sodium (mg/person/day) from SFC <sup>b</sup>		Estimated Intake of Sodium (mg/person/day) <sup>c</sup>	
		Mean (mg/person/day)	90 <sup>th</sup> percentile (mg/person/day)	Mean (mg/person/day)	90 <sup>th</sup> percentile (mg/person/day)	Mean (mg/person/day)	90 <sup>th</sup> percentile (mg/person/day)
Children	1 to 3 years	10.36	17.60	17.53	29.78	2,114	3,403
	4 to 8 years	14.68	21.27	24.84	36.00	2,864	3,636
Males	9 to 13 years	18.05	25.70	30.55	43.49	3,809	4,950
	14 to 18 years	20.88	32.68	35.34	55.30	4,598	6,127
	19 to 30 years	20.87	31.84	35.32	53.88	4,746	5,539
	31 to 50 years	21.09	33.48	35.69	56.66	4,418	6,187
	51 to 70 years	20.64	34.30	34.93	58.05	3,781	5,100
	>70 years	20.95	34.50	35.45	58.38	3,198	4,508
Females	9 to 13 years	14.63	21.84	24.76	36.96	3,178	4,160
	14 to 18 years	13.24	19.61	22.41	33.19	3,083	4,592
	19 to 30 years	16.76	29.10	28.36	49.25	3,159	3,978
	31 to 50 years	17.11	31.01	28.96	52.48	3,032	4,146
	51 to 70 years	16.83	30.46	28.48	51.55	2,613	3,644
	>70 years	19.01	32.03	32.17	54.20	2,395	3,504
All individuals, including pregnant and lactating females		18.34	30.13	31.04	50.99	3418	5120

<sup>a</sup> IOM, 2001

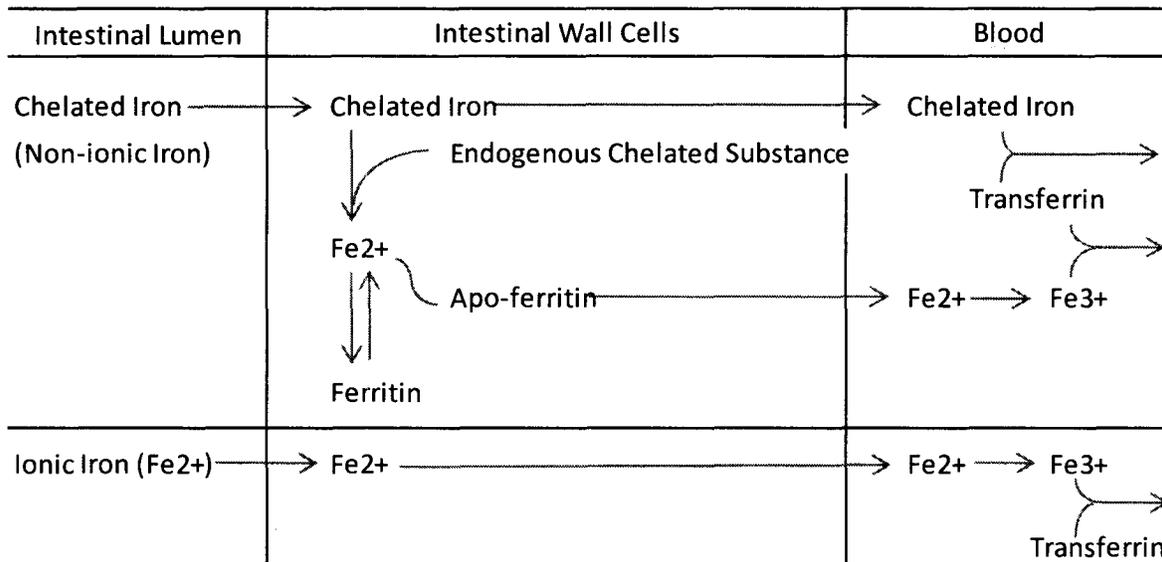
<sup>b</sup> Calculated value based on the assumption that all iron intake is a result of SFC consumption; Iron comprises 10.4% SFC; Sodium comprises 17.6% SFC

<sup>c</sup> IOM, 2004

**C. Metabolic Fate**

SFC is a complex of sodium, citric acid, and iron(II) that is primarily used as a dietary source of iron, but also provides sodium and citric acid/citrates based on its absorption characteristics. In the acidic conditions of the upper gastrointestinal tract, organic iron, as is the case of iron from SFC, is considered to be absorbed from the small intestine (primarily the duodenum) in 2 ways. The first as chelated iron consisting of iron and citric acid and the second as dissociated ionic iron (Helbock and Saltman, 1967; Spiro and Saltman, 1967; Fujita and Terato, 1973; Terato *et al.*, 1973). During transportation from the mucosa to serosa in the small intestine some unstable chelated iron is dissociated through the presence of endogenous chelating agents and apo-ferritin. The iron from the dissociated chelate is then transferred into the circulatory blood supply, while stable chelated iron is transferred as is, as shown in Figure III.C-1 below.

**Figure III.C-1 Absorption of Sodium Ferrous Citrate**



(Cited from G. Spiro and P. Saltman: Springer-Verlog Berlin Heiderberg N.Y. 1967)

Iron from stable chelated iron is considered also to be absorbed into the blood stream where the citric acid is replaced with transferrin in the chelated iron because the affinity of iron to transferrin is stronger than that to citric acid. The iron is bound to transferrin to be utilized as a nutrition source. Any sodium and citrate released from the parent compound will be absorbed by the gastrointestinal tract and utilized in the body accordingly. Therefore, the formation of the individual ions may be considered separately when assessing the overall metabolic fate of SFC in addition to the parent compound *per se*.

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### C.1 Sodium

Dietary sodium is primarily absorbed (98%) in the small intestine and excreted in the urine at levels approximately equal to intake (IOM, 2004). The human kidney has the capacity to filter 25,000 mmol sodium per day and reabsorbs at least 99% of the filtered load (Valtin and Schafer, 1995). Sodium may also be excreted through the feces, albeit at minimal levels (*e.g.*, less than 5% of sodium was excreted *via feces* in males with intakes of 8 g sodium/day). Sodium also may be lost to sweat and this loss is dependent on sweat rate, sodium intake, and heat acclimation (IOM, 2004).

Once absorbed, sodium is mainly present in the extracellular fluid, including plasma, interstitial fluid, and plasma water. Sodium concentrations in these compartments are in the 100 mmol/L range, whereas intracellular concentrations are 2 orders of magnitude lower. The concentration gradient is maintained by the Na<sup>+</sup>K<sup>+</sup>-ATPase pump. Sodium homeostasis is influenced by various systems and hormones, including the renin-angiotensin-aldosterone axis, the sympathetic nervous system, atrial natriuretic peptide, the kallikrein-kinin system, several intrarenal mechanisms, and other factors regulating renal and medullary blood flow.

### C.2 Citrates/Citric Acid

Citrates/citric acid are well absorbed *via* the oral route and are involved in oxidative metabolism through the citric acid cycle (*a.k.a.* Kreb's cycle) (Nelson and Cox, 2000).

### C.3 Iron/Sodium Ferrous Citrate

The absorption characteristics of iron have been shown to vary according to physiological need. Among 18 male volunteers stratified according to iron status at baseline (above or below serum iron concentration of 118 µg/dL) that were provided 2 gastric- or enteric-coated tablets (100 mg iron from SFC) in a crossover design, the maximum increment of serum iron concentration was greatest among individuals with a lower iron status at baseline (94.1 and 86.6 vs. 37.6 and 7.9 µg/dL for individuals consuming the gastric and enteric tablets in the low vs. high iron status groups, respectively); however, the time to reach the maximum concentration ( $T_{max}$ ) was shorter among individuals with higher baseline iron values (5.1 and 7.8 vs. 2.5 and 2.7 hours for individuals consuming the gastric and enteric tablets in the low vs. high iron status groups, respectively) (Miyao *et al.*, 1984). The absorption of iron also is influenced by the solubility of the iron salt. Generally, iron from ferrous salts, such as SFC, are more readily absorbed than iron from ferric salts (JECFA, 1983). Kobune *et al.* (2011) demonstrated that the absorption of iron was higher among individuals provided tablets containing 100 mg iron from SFC (n=17) in comparison to iron from ferrous sulfate (n=6), with the serum iron concentration in individuals administered SFC and ferrous sulfate reported to be approximately 150 and 125 µg/dL 180 minutes post-dosing, respectively.

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As mentioned previously, organic iron from SFC is absorbed from the small intestine (primarily the duodenum) in 2 ways. The first as chelated iron consisting of iron and citric acid and the second as dissociated ionic iron (Spiro and Saltman, 1967; Forth and Rummel, 1973; Fujita and Terato, 1973; Terato *et al.*, 1973). The dissociated iron is then transferred into the circulatory blood supply, where it is oxidized to its ferric form and bound to transferrin (JECFA, 1983; INACG, 1993; IOM, 2001; EVM, 2002). Through the binding of transferrin to the transferrin receptor of cell membranes, iron is released into cells throughout the body (INACG, 1993; IOM, 2001; EVM, 2002). Funahashi *et al.* (1986) measured serum iron levels in dogs (5/sex/group) following oral administration of 50, 150, or 450 mg SFC/kg body weight [equivalent to 5, 15, or 45 mg iron(II)/kg body weight] and observed that the  $T_{max}$  was recorded between 1 and 2 hours after administration. Additionally, the authors indicated that the  $C_{max}$  did not increase at doses greater than 150 mg SFC/kg body weight ( $C_{max}$  approximately 0.340, 0.440, and 0.440 mg/dL after the first oral administration in the 50, 150, or 450 mg/kg body weight dose groups, respectively), indicating a saturation of absorption mechanisms (Funahashi *et al.*, 1986). Ariyoshi *et al.* (1987) administered  $^{59}\text{Fe}$ -SFC or  $^{59}\text{FeSO}_4$  to Sprague-Dawley rats (3/group) as a single dose of 0.75 or 1.5 mg iron(II)/kg body weight, respectively, via the oral route and determined that the blood concentration of radioactivity in the  $^{59}\text{Fe}$ -SFC group peaked at both 1 hour and 48 hours post-administration. In the  $^{59}\text{FeSO}_4$  group, however, blood radioactivity levels peaked at 1 hour post-administration and then steadily increased, reaching a plateau at 48 hours after administration.  $C_{max}$  at 48 hours in the  $^{59}\text{Fe}$ -SFC group was higher than the  $^{59}\text{FeSO}_4$  group, (blood concentrations were approximately 1,600 vs. 1,050 ng/mL for  $^{59}\text{Fe}$ -SFC and  $^{59}\text{FeSO}_4$  groups, respectively) and indicate a greater absorption of iron from SFC. In a similar study, Arizono *et al.* (1996) evaluated the absorption of orally administered  $^{59}\text{Fe}$ -SFC or  $^{59}\text{FeSO}_4$  within lactating Sprague-Dawley rats (3 to 5/group) following a single dose of 7.5 mg iron(II)/kg body weight. The concentration of radioactive iron in the plasma was 4.6-fold higher in the  $^{59}\text{Fe}$ -SFC group compared to the  $^{59}\text{FeSO}_4$  group at 1 hour post-administration and remained significantly higher 6 hours after administration (Arizono *et al.*, 1996). The results of Arizono *et al.* (1996) and Ariyoshi *et al.* (1987) support the greater absorption of SFC compared to  $\text{FeSO}_4$ . Due to this increased absorption of SFC, the required intake to achieve comparable plasma concentrations of iron would likely be lower than other iron salts.

If iron is present in the body in excess, it will be stored as ferritin and hemosiderin in the liver, bone marrow, and reticuloendothelial cells. Several proteins such as hemoglobin, myoglobin, and cytochromes require iron for biological function (INACG, 1993; IOM, 2001; EVM, 2002). An increase in radioactivity in red blood cells among male Sprague-Dawley rats orally administered 0.75 or 1.5 mg iron(II)/kg body weight, as  $^{59}\text{Fe}$ -SFC or  $^{59}\text{FeSO}_4$ , respectively, as observed by Ariyoshi *et al.* (1987), indicates that iron from SFC is incorporated into hemoglobin. In the  $^{59}\text{Fe}$ -SFC group, the concentration of radioactivity in the red blood cells was approximately 1.7-fold higher and increased more rapidly in comparison to the rats orally administered  $^{59}\text{FeSO}_4$ . These observations are consistent with the findings of Arizono *et al.* (1996) wherein the maximum level of radioactivity in red blood cells of lactating rats was 3-fold greater in the

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<sup>59</sup>Fe-SFC group compared to the <sup>59</sup>FeSO<sub>4</sub> group, after oral administration. High levels of radioactivity were observed in the liver, spleen and bone of lactating rats 24 hours after <sup>59</sup>Fe-SFC oral administration (Arizono *et al.*, 1996) and in tissues, including the liver, spleen, and bone of male rats (Ariyoshi *et al.*, 1987). These observations demonstrate the rapid incorporation of iron from SFC into the hemoglobin of red blood cells, as well as tissues such as liver, spleen, and bone, and further support efficient absorption of iron from SFC.

The excretion of iron is influenced by the homeostatic degradation process of erythrocytes, as nearly 100% of the iron utilized is recycled into new erythrocytes (INACG, 1993; IOM, 2001; EVM, 2002). As a result, only small amounts of iron are excreted each day, unless certain physiological distresses, such as blood loss, occur. The primary route for excretion of iron is through feces; however, small amounts of iron may be excreted through the urine, desquamated gastrointestinal cells, and bile. The excretion of <sup>59</sup>Fe in the feces was reported by Ariyoshi *et al.* (1987) to be significantly lower in the <sup>59</sup>Fe-SFC group compared to the <sup>59</sup>FeSO<sub>4</sub> group (39.27±2.22 vs. 53.88±0.92% of the administered dose, respectively) and low amounts of excretion of <sup>59</sup>Fe in the urine (0.33±0.12 and 0.32±0.10% of the administered dose, respectively) is indicative of iron utilization in the body.

### D. Safety Aspect of Individual Components

#### D.1 Sodium

Based on the effects of sodium on blood pressure, the IOM established the UL for sodium to range from 1.5 to 2.2 g/day for children, and 2.3 g/day for adolescents and adults (IOM, 2004). Based on the proposed uses of SFC, the highest worst-case scenario mean and 90<sup>th</sup> percentile intakes of sodium were estimated to be 35.7 mg/person/day for males 31 to 50 years of age and 58.4 mg/person/day for males greater than 70 years of age, respectively. These levels are well below the UL for sodium.

#### D.2 Citrate

Citric acid is affirmed as GRAS for use in food with no limitations other than cGMP (21 CFR §184.1033) (U.S. FDA, 2011). Furthermore, the safety of citric acid was evaluated by JECFA at the 17<sup>th</sup> meeting (JECFA, 1974). An acceptable daily intake (ADI) of “not limited” was determined by JECFA due to its well established role in oxidative metabolism and its lack of significant toxicological risk to human health.

#### D.3 Iron

The safety of iron has previously been evaluated by JECFA and the IOM (JECFA, 1983; IOM, 2001). Following the 27<sup>th</sup> meeting, JECFA established a provisional maximum tolerable daily intake (PMTDI) of 0.8 mg iron/kg body weight for all iron sources in normal individuals (*e.g.*,

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without blood related diseases, pregnant or breast feeding women) except iron oxides used in coloring agents, equivalent to a daily dose of 56 mg for a 70 kg individual (JECFA, 1983). Additionally, JECFA maintained the PMTDI following recent reviews of other iron salts (JECFA, 2008). The IOM derived an UL for iron of 45 mg/person/day for adolescents and adults based on findings of gastrointestinal side effects at 70 mg/person/day [lowest-observed-adverse-effect level, (LOAEL)] and using an uncertainty factor of 1.5 to extrapolate the LOAEL to a no-observed-adverse-effect level (NOAEL).

The median oral LD<sub>50</sub> values of iron from ferrous sulfate in mice, rats, rabbits, and dogs are approximately 670, 344, 200, and 200 mg/kg body weight, respectively (Keith, 1957; Boccio *et al.*, 1998; Whittaker *et al.*, 2002). Consistent adverse effects were noted among laboratory animals following acute exposure and included gastrointestinal disturbances such as vomiting and diarrhea, as well as lesions in the stomach and proximal small intestine. Following repeated dietary iron administration, the adverse effects observed in rodents were hepatic in nature and included increased hepatic iron content and deposition, serum indicators of liver toxicity, and hepatic microsomal lipid peroxidation at doses of up to 1,500 mg elemental iron/kg body weight/day as elemental iron for up to 12 weeks (Wu *et al.*, 1990; Omara and Blakley, 1993; Omara *et al.*, 1993; Stål and Hultcrantz, 1993; Olynyk *et al.*, 1995; Stål *et al.*, 1996; Whittaker *et al.*, 1996; Hirohata *et al.*, 1998; Appel *et al.*, 2001). Increases in serum indicators of liver toxicity and hepatic microsomal lipid peroxidation, however, were not typically accompanied by macro- or microscopic histopathological abnormalities, including necrosis or fibrosis. Exposure to carbonyl iron (up to 4,500 mg elemental iron/kg body weight/day) for up to 1 year did not result in the development in fibrosis, cirrhosis, or tumors in rodents (Iancu *et al.*, 1987; Park *et al.*, 1987; Plummer *et al.*, 1997; Pigeon *et al.*, 1999). Additionally, maternal toxicity or teratogenic effects were not observed following consumption of iron at doses of up to 75 mg iron(II)/kg body weight/day, provided as ferrous sulfate in mice and rats (JECFA, 1983; Fairweather-Tait *et al.*, 1984). At high concentrations [up to 10,000 mg iron(II)/plate], iron(II) has no or very low genotoxic potential as confirmed by *in vitro* mutagenicity assays (Casto *et al.*, 1979; Dunkel *et al.*, 1999).

Acute iron toxicity has been shown to occur in humans as a result of accidental poisoning (McGuigan, 1996). At dosages above 50 mg/person/day, repeated oral iron supplementation may lead to adverse gastrointestinal effects (*e.g.*, abdominal distension, constipation, diarrhea) (Hallberg *et al.*, 1966; Brock *et al.*, 1985; Laine *et al.*, 1988; Coplin *et al.*, 1991; Liguori, 1993; IOM, 2001); however, these adverse events were related to the consumption of iron supplements and not to iron fortification of foods. It may be concluded from a critical evaluation of the toxicological potential of iron that the risk of iron overload and any associated adverse effects as a result of the consumption of foods fortified with iron is very low. Findings from human studies indicate that supplementation with iron is not teratogenic and does not result in adverse birth outcomes or adverse growth and development, and thus, is safe to the developing

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fetus and/or infant (Preziosi *et al.*, 1997; Cogswell *et al.*, 2003; Berseth *et al.*, 2004; Siega-Riz *et al.*, 2006; Zhou *et al.*, 2006; Abdelrazik *et al.*, 2007; Steinmacher *et al.*, 2007).

### **E. Toxicological Studies Conducted with Sodium Ferrous Citrate**

A number of preclinical studies investigating the safety of SFC were conducted, including acute and repeat-dose animal feeding studies, reproductive and developmental toxicology studies, and short-term genotoxicity studies. Consistent with the noted iron-related effects following acute and repeated administration with other GRAS affirmed iron compounds (primarily ferrous sulfate), administration with SFC at equivalent dosages, resulted in similar gastrointestinal disturbances (vomiting, diarrhea), lesions in the gastrointestinal tract, and iron deposition in the liver and other organs. Therefore, given no other untoward adverse effects, the available studies conducted with SFC further corroborate the conclusions from previous scientific reviews on iron (IOM, 2001, JECFA, 2008). The results of the available preclinical studies on SFC are discussed below.

#### **E.1 Acute Studies**

Oral LD<sub>50</sub> values for SFC in laboratory animals were determined to be 2,400 (male) or 4,100 (female) [equivalent to 240 or 410 mg iron(II)/kg body weight, respectively], 4,200 (male) or 4,000 (female) [approximately 420 or 400 mg iron(II)/kg body weight, respectively], and ≥5,000 [≥500 mg iron(II)/kg body weight], in the mouse, rat, and dog, respectively (Sumikama *et al.*, 1985; Funahashi *et al.*, 1986). Similar to the adverse effects noted in acute studies conducted with other iron preparations (*e.g.*, ferrous fumarate, ferrous sulfate), as noted in Section D.3, gastrointestinal disturbances (*e.g.*, diarrhea, black contents in the intestinal tract, hemorrhage and edema in the glandular stomach, and discoloration of the liver) were observed; therefore, the effects are not considered to be specific to SFC.

#### **E.2. Repeat-Dose Studies**

In a short-term study comparing the effects of SFC and ferrous sulfate following oral administration, male Sprague-Dawley rats (8/group) were administered either iron source by gavage at doses of 0 (control), 150, or 250 mg iron(II)/kg body weight/day for 3 or 7 days, 1 hour after administration of a histamine H<sub>2</sub>-blocker (Fujimori and Taki, 1987). The types of adverse effects observed among animals administered either iron source were similar and included iron deposition, thickening of the mucosa, and mild inflammation and ulceration in the proventriculus and glandular stomach; however, the effects observed within the SFC groups were noted to be milder than those associated with the dosing of ferrous sulfate. Also, the gastric injury index was reported to be significantly lower in the SFC groups compared to the ferrous sulfate groups.

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Among Sprague-Dawley rats (10/sex/group) administered SFC at doses of 0 (control), 300, 1,000, 3,000, or 5,000 mg/kg body weight/day [equivalent to 0, 30, 100, 300, or 500 mg iron(II)/kg body weight/day] for 35 days by gavage, adverse effects commonly associated with high iron intakes, including decreases in body weight gains associated with decreases in food intake and diarrhea were observed in groups administered greater than or equal to 3,000 mg SFC/kg body weight/day (Mitsuzono *et al.*, 1985). Three rats administered 5,000 mg/kg on day 2 of treatment suffered pulmonary congestion, gastric distension with gas and diarrhea leading to death. The cause of death was not clarified but was considered by the authors to be related to the large dose of SFC leading to circulatory disturbance and gastrointestinal dysfunction. No abnormal hematology findings were reported across all dose groups. In contrast, decreases in plasma and urine electrolyte concentrations (calcium, chlorine and potassium) and occult blood and ketonuria were detected at 3,000 mg/kg/day and higher. The electrolyte changes were considered by the authors to be caused by the diarrhea and increase in water intake during treatment. Compound-related increases in urinary pH were considered by the study authors to be due to the alkalinizing effect of citrate and the increased sodium concentration in the urine was indicated to be due to the sodium released from SFC during metabolism. Similar to repeat-dose studies conducted with other iron fortificants, the observed increases and decreases in relative and/or absolute organ weights (liver, kidney thymus, and testes) were reported to be slight. As a result of the above observations, and taking into account the individual components (sodium, iron and citrate) the authors indicated that a maximum safety level for SFC of 1,000 mg/kg body weight/day (equivalent to 70 g SFC/day for a 70 kg individual or approximately 7 g iron/day) could be determined (Mitsuzono *et al.*, 1985). In comparison to the UL for iron presented in Table IV.B.1-1, the maximum safety level is more than 155-fold higher than the adult UL established by the IOM.

Similar to the effects observed in rats, diarrhea observed among Beagle dogs (5/sex/group) administered doses of SFC by gavage greater than or equal to 150 mg/kg body weight/day [equivalent to  $\geq 15$  mg iron(II)/kg body weight/day] for 13 weeks was attributed to the administration of SFC (Funahashi *et al.*, 1986). In contrast, no effects were noted on bodyweight, food consumption or water intake and while some significant differences were noted within hematology and blood chemistry parameters these all fell within physiological variation. Increased hemosiderin deposits in dogs were observed in the liver, kidney, spleen, thymus, submaxillary lymph nodes, tonsil, and gastrointestinal tract in the mid and high dose groups [150 and 450 mg SFC/kg body weight/day, equivalent to 15, and 45 mg iron(II)/kg body weight/day]. The authors noted that the deposition was most severe in the highest dose group and that the amount of iron absorbed increased through the GI tract as the dose increased and that the excess iron was stored in these organs. The hemosiderin deposits also decreased during the wash out period. Such findings are consistent with the findings from repeat-dose studies conducted with other iron sources (Papanastasiou *et al.*, 2000). Funahashi *et al.* (1986) reported a NOAEL of 50 mg/kg body weight/day in dogs due to the hemosiderosis; however, it was indicated by the authors that storage of excess iron was not damaging to organ function, as

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demonstrated by the lack of effects observed within hematological, clinical chemistry, and urine analyses. The NOAEL derived for the dog is equivalent to 3.5 g SFC/day for a 70 kg individual, or approximately 350 mg iron/day, and is about 8-fold greater than the adult UL for iron.

### E.3 Developmental and Reproductive Toxicity Studies

Okada *et al.* (1988) evaluated the teratogenic and reproductive effects of SFC. Similar to the lack of maternal or developmental toxicities reported in studies conducted with mice and rats administered doses of up to 75 mg iron(II)/kg body weight/day as ferrous sulfate, as reviewed by JECFA (1983), no maternal or developmental adverse effects were observed among pregnant Japanese white rabbits (16/group) administered SFC by gavage on Day 6 to 18 of gestation at doses of 100 or 300 mg/kg body weight/day [equivalent to 10 or 30 mg iron (II)/kg body weight/day, respectively]. However, at very high doses of SFC [1,000 mg/kg body weight/day, equivalent to 100 mg iron(II)/kg body weight/day], moderate to severe edematous degeneration in the renal cortical tubular epithelial cells, mild to moderate shedding of the brush border of proximal tubule epithelial cells, mild iron staining in the superficial mucous cells of the stomach, and a non-statistically significant increase in the incidence of spontaneous abortions attributed to malnutrition (decreases in food intake accompanied by a decrease in body weight and diffuse hepatocyte vacuolation) were observed. Overall, the authors reported the NOAEL for reproduction to be 300 mg/kg body weight/day (equivalent to 21 g SFC/day based on a 70 kg individual, or approximately 2.1 g iron/day) based on the spontaneous abortions associated with decreased food intake, and due to the effects observed in the kidney and stomach observed at higher doses. This NOAEL is more than 45-fold higher than the UL for iron established for adults. As all of the effects in the fetuses were determined not to be biologically significant, the NOAEL for offspring was determined to be 1,000 mg/kg body weight/day, which is equivalent to 20 g SFC/day for a 20 kg child, or 2 g iron/day, and is 50-fold higher than the UL for iron for children.

### E.4 Short-Term Tests for Genotoxicity

Reverse mutation assays were conducted in *Salmonella typhimurium* TA1535, TA1537, TA1538, TA98, and TA100 and *Escherichia coli* WP2/uvr A, and DNA repair assays were conducted in *Bacillus subtilis* H17 (*rec*<sup>+</sup>) and M45 (*rec*<sup>-</sup>) and *E. coli* W3110 (*pol. A*<sup>+</sup>) and p3478 (*pol. A*<sup>-</sup>) at concentrations up to 5,000 µg/disc (Mochida *et al.*, 1986). Since both the reverse mutation test and DNA repair test were observed to be negative in the presence or absence of metabolic activation (S-9), it was concluded that SFC was unlikely to possess mutagenic activity.

## F. Human Studies

The oral administration of SFC was examined in 3 identified human studies although reported results were primarily related to efficacy (Miyao *et al.*, 1984; Imamura and Kuramoto, 1987;

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Shirakura *et al.*, 1988). Similar to human studies conducted with other iron fortificants, as presented in Section D.3, adverse effects reported following consumption of SFC at doses up to 200 mg iron(II)/day for up to 37 weeks were related to gastrointestinal disturbances such as constipation, diarrhea, abdominal discomfort, and abdominal pain. These doses are more than 4-fold higher than the adult UL for iron established by the IOM of 45 mg/person/day; therefore, the supplementation with SFC does not present any additional safety concerns in comparison to other GRAS affirmed iron substances.

Miyao *et al.* (1984) reported subjective symptoms, such as constipation, diarrhea, heartburn, nausea, anorexia, abdominal discomfort, and abdominal pain, after oral consumption of SFC (100 or 200 mg as iron) administered daily for 14 days to male volunteers, although the significance of incidence of the effects were not reported. It should be noted that the incidence of these effects appear to be similar between the groups. These adverse effects, however, were reported to be transient and disappeared within 2 to 3 hours after onset, with additional occurrences of these symptom reported for 3 to 4 days and no reports thereafter. Significant changes in serum iron, serum iron saturation, serum ferritin, reticulocyte, ratio of reticulocyte production index, and mean corpuscular hemoglobin concentration (MCHC) also were reported to support the treatment of anemia, while having no safety concern. Furthermore, no significant effects on serum biochemical parameters, including serum electrolytes (sodium, potassium, chloride, and calcium) were observed at either dosage.

Shirakura *et al.* (1988) reported that 10 of a total of 94 subjects administered a jelly containing 20 mg SFC/day (approximately 2 mg iron/day) for 30 or 60 days complained of gastric symptoms (*e.g.*, stomach ache and heavy feelings in the stomach) immediately after the beginning of treatment, lasting for several days. Of these 10 subjects experiencing gastric symptoms, 7 withdrew from the study but subsequently returned to complete the study with no further complaints of gastric symptoms occurring. Although significance was not reported, the authors indicated that a causal relationship between the administration of SFC and the gastric symptoms was not clear (Shirakura *et al.*, 1988). Significant increases in serum iron, transferrin saturation, and serum ferritin as well as significant decreases in total iron binding capacity, also were reported and although these effects are indicative of efficacy for the treatment of anemia, the changes were of no safety concern.

In the study by Imamura and Kuramoto (1987), no cases of aggravated anemia were reported in iron deficient anemic volunteers that were administered SFC at doses of 100 or 200 mg iron(II)/day for 5 to 37 weeks. Subjective side effects (*e.g.*, gastric effects) were not reported in 58.8 and 62.5% of the subjects in the 100 or 200 mg iron(II)/day groups, respectively, and of the effects that were reported, all were considered to be mild and included constipation, diarrhea, abdominal discomfort, nausea, and vomiting. All subjects completed treatment. Additionally, significant differences in hemoglobin levels were not observed between the 100 and 200 mg iron(II)/day groups. There were no abnormal findings in hematological examinations, clinical

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chemistry including serum electrolytes (sodium, potassium, chloride, and calcium), or urinalysis in volunteers receiving SFC for 10 weeks or longer.

### **G. Summary and Basis for GRAS Conclusion**

The GRAS determination for the use of SFC as a food ingredient is based on scientific procedures. Eisai intends to market SFC as a direct replacement for other iron salts in existing categories of fortified foods currently marketed in the U.S. at levels consistent with cGMP in accordance with the Fortification Policy outlined in the Nutritional Quality Guidelines for Foods set forth by the FDA (21 CFR §104.20) (U.S. FDA, 2011). Based on the assumption that SFC would replace all dietary forms of iron in foods and supplements, the worst-case intake scenario of SFC was determined based on the estimated intake of iron from foods and supplements by the IOM. The mean intake of iron is 18.34 mg/person/day for all individuals, including pregnant and lactating females whereas the 90<sup>th</sup> percentile intakes of iron is 30.13 for all individuals, including pregnant and lactating females. The potential intake of sodium from SFC was extrapolated from the estimated iron intake based on the sodium content of SFC (17.6%). The mean calculated intake of sodium from SFC for all age groups is 31.04 mg/person/day, whereas the calculated 90<sup>th</sup> percentile intake of sodium for all age groups is 50.99 mg/person/day. These values are more than 100 times lower than the mean and 90<sup>th</sup> percentile dietary sodium intakes estimated by the IOM. Overall, the worst-case 90<sup>th</sup> percentile intake scenarios of iron and sodium are more than 1.3 and 29 times lower than the ULs of 40 and 1500 mg/person/day for iron and sodium, respectively.

SFC is manufactured in accordance with cGMP from trisodium citrate and ferrous sulfate and meets appropriate food grade specifications. The material is well characterized and consists of sodium, citric acid, and iron(II) in a molar ratio of 4:2:1, respectively. Lot samples are routinely assayed to verify compliance with specifications. Stability studies demonstrate that SFC was satisfactorily stable under normal conditions.

Information to support the general recognition of the safe use of SFC is based on the opinions of internationally acclaimed scientific and regulatory agencies on the safety of sodium, citrate, and iron, the metabolic fate of SFC and its individual components, and preclinical and human studies conducted with SFC further corroborating the authoritative reviews.

The safety of sodium, citrate, and iron has been previously evaluated by other internationally acclaimed scientific and regulatory agencies, including JECFA, the IOM, and the FDA. The IOM established an UL for sodium with a range of 1.5 to 2.2 g/day for children, and 2.3 g/day for adolescents and adults (IOM, 2004). JECFA determined an ADI of "not limited" for citric acid due to its well established role in oxidative metabolism and its lack of significant toxicological risk to human health (JECFA, 1974) and established a PMTDI of 0.8 mg iron/kg body weight in normal individuals, equivalent to a daily dose of 56 mg for a 70 kg individual (JECFA, 1983).

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Similarly, an UL for iron of 45 mg/person/day for adolescents and adults was derived by the IOM (2001).

SFC is a dietary source of iron that has been shown to be absorbed in the upper gastrointestinal tract, mainly in the duodenum. Accordingly, SFC also provides sodium and citric acid/citrates as dietary sources based on the absorption mechanism of SFC. Sodium is known to be almost entirely absorbed in the small intestine and excreted in the urine at concentrations equal to intake, whereas citric acid is well absorbed and is actively involved in the citric acid cycle. Citric acid is a natural component in the diet and is regarded as a normal metabolite in the body. Likewise, organic iron from SFC is considered to be absorbed into the intestinal mucosa of the small intestine in 2 ways, with the metabolic fate dependent on physiological need: the first as chelated iron consisting of iron and citric acid, and the second as dissociated ionic iron. Some iron from SFC is absorbed into the systemic circulatory system as ionic iron, while some stable chelated iron is absorbed, as is, into the blood stream where it dissociates and the iron binds to transferrin. Iron is then used in proteins in the body that require iron for biological function. If iron is in excess and not utilized at any given time, it is stored as ferritin and hemosiderin in the liver, bone marrow, and in reticuloendothelial cells. Within the homeostatic degradation process of erythrocytes, iron is largely recycled into new erythrocytes and thus, only small levels of iron are excreted each day under normal conditions. Small amounts of iron also may be excreted *via* the urine, desquamated gastrointestinal cells, and bile.

Acute oral toxicity studies conducted in the mouse, rat, and dog established LD<sub>50</sub> values in the range of to be 2,400 to  $\geq 5,000$  mg SFC/kg body weight [equivalent to 240 to  $\geq 500$  mg iron(II)/kg body weight, respectively], with the reported adverse effects (diarrhea, mild sedation, and discoloration in the liver) shown to be consisted with other iron sources (Sumikama *et al.*, 1985; Funahashi *et al.*, 1986). Similarly, the repeated administration of SFC in rats and dogs was associated with diarrhea at doses  $\geq 3,000$  and  $\geq 150$  mg/kg body weight/day, respectively (equivalent to  $\geq 300$  and  $\geq 15$  mg iron(II)/kg body weight/day, respectively) and decreases in food intake and body weight gains in male rats administered 3,000 mg and female rats administered 5,000 mg SFC/kg body weight/day, both of which are considered to be common effects associated with high intakes of iron (Mitsuzono *et al.*, 1985; Funahashi *et al.*, 1986). Likewise, the observed increases and decreases in relative and/or absolute organ weight (liver, kidney, thymus, and testes) were reported to be slight. The changes in rat urine pH were considered to occur as a result of the citrate. Furthermore, another study, showed inflammation, erosion, ulceration, and iron deposition in the stomach of rats administered SFC or ferrous sulfate at 150 or 250 mg/kg body weight/day for 3 or 7 days; however, these effects were reported by the authors to be less severe in the SFC groups compared to the ferrous sulfate groups (Fujimori and Taki, 1987).

No maternal or developmental toxicities were observed in a reproductive toxicity study conducted in Japanese white rabbits (Okada *et al.*, 1988). Cases of spontaneous abortions at

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high doses (*i.e.*, 1,000 mg/kg body weight/day), which were not statistically significant however, could not be dismissed but were considered to result from decreased food intake and a subsequent decrease in bodyweight. Moreover, identified mutagenicity studies for SFC all indicated negative results (Mochida *et al.*, 1986). In comparison to toxicity studies conducted with other iron compounds (*e.g.*, ferrous sulfate) at high levels, the results observed in preclinical studies with SFC (*i.e.*, gastrointestinal disturbances) are similar in terms of the type of adverse reactions but lower in their incidence and severity. Likewise, human studies following oral consumption of SFC (100 or 200 mg iron/day for up to 14 days, 100 or 200 mg iron/day for 5 to 37 weeks, or 2 mg iron/day for up to 60 days) did not result in serious adverse effects (Miyao *et al.*, 1984; Imamura and Kuramoto, 1987; Shirakura *et al.*, 1988). Furthermore, a study conducted by Imamura and Kuramoto (1987), demonstrated that SFC was without clinically significant side effects following treatment for iron deficiency anemia. No hematological or biochemical significant adverse effects were reported in those patients that received SFC for a period of 10 weeks or longer.

Together, the above data support the conclusion that the consumption of SFC under the intended conditions of use would not be expected to produce adverse effects differing from other dietary iron sources that are already recognized as being GRAS, and that these potential adverse effects are expected to be minor and less severe than other dietary iron sources.

Finally, the Expert Panel convened on behalf of Eisai, independently and collectively, critically evaluated the data and information summarized above and concluded that the intended uses of SFC, produced consistently with cGMP and meeting appropriate food-grade specifications described herein, are safe and GRAS based on scientific procedures. It also is the Expert Panel's opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. SFC is GRAS based on scientific procedures for its intended use as a nutritive ingredient, specifically a dietary source of iron; therefore, it is excluded from the definition of a food additive and thus may be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21 of the CFR.

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<b>Table of CFR Sections Referenced (Title 21—Food and Drugs)</b>		
<b>Part</b>	<b>Section §</b>	<b>Section Title</b>
101—Food labeling	101.9	Nutrition labeling of food
104—Nutritional quality guidelines for foods	104.20	Statement of Purpose
170—Food additives	170.30	Eligibility for classification as generally recognized as safe (GRAS)
184—Direct food substances affirmed as generally recognized as safe	184.1	Substances added directly to human food affirmed as generally recognized as safe (GRAS)
	184.1033	Citric acid
	184.1315	Ferrous sulfate
	184.1751	Sodium citrate

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## **Appendix A**

### **Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of Sodium Ferrous Citrate for Use as a Food Ingredient**

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# Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of Sodium Ferrous Citrate for Use as a Food Ingredient

October 28, 2011

## INTRODUCTION

At the request of Eisai Food & Chemical Co., Ltd. (herein "Eisai"), an Expert Panel (the "Panel") of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information, and determine whether the intended uses of sodium ferrous citrate (SFC) as a food ingredient are safe and suitable and would be Generally Recognized as Safe (GRAS) based on scientific procedures. The Panel consisted of: Dr. John Doull, Ph.D., M.D. (University of Kansas Medical Center), Dr. Robert J. Nicolosi, Ph.D. (University of Massachusetts Lowell), and Dr. Stephen L. Taylor, Ph.D. (University of Nebraska).

The Panel, independently and collectively, critically examined a comprehensive package of scientific information and data on SFC from the literature and other published sources through October 2011. This information was presented in a dossier [**Documentation Supporting the Evaluation of Sodium Ferrous Citrate as Generally Recognized as Safe (GRAS) for Use as a Food Ingredient**] that was submitted by Eisai to the Panel. In addition, the Panel evaluated other information deemed appropriate or necessary. The information evaluated by the Panel included details pertaining to the method of manufacture and product specifications, supporting analytical data, intended use-levels in specified food products, consumption estimates for all intended uses, and a comprehensive assessment of the available scientific literature pertaining to the safety of SFC.

Following independent, critical evaluation of such data and information, the Panel convened on October 28, 2011 *via* teleconference and unanimously concluded that the intended uses in traditional foods described herein for SFC, meeting appropriate food-grade specifications as described in the supporting dossier and manufactured according to current Good Manufacturing Practice (cGMP), are safe and suitable and GRAS based on scientific procedures. A summary of the basis for the Panel's conclusion is provided below.

## SUMMARY AND BASIS FOR GRAS

SFC is manufactured in accordance with cGMP *via* a chemical reaction between trisodium citrate and ferrous sulfate. The material is well characterized and consists of sodium, citrate, and iron(II) in a molar ratio of 4:2:1, respectively. Details regarding the technical data for SFC (*i.e.* composition of SFC, and chemical and physical characteristics) are described by Ishino *et al.* (1988). Specifications and analytical methods for SFC have been established based on the 8<sup>th</sup> Edition of Japanese Standards of Food Additives for SFC (MHLW, 2009). Additionally, analyses for lead and microbial contaminants have been established to demonstrate that SFC meets appropriate food-grade specifications. Lot samples are routinely assayed to confirm that SFC is in compliance with the specifications. Ishino *et al.* (1988) also described the stability of SFC under various conditions (*i.e.*, heat, humidity, light in powder form, in aqueous solutions, in tablets, as granules) and determined that SFC was satisfactorily stable under normal conditions.

Eisai intends to market SFC as a direct replacement to other iron salts in existing categories of fortified foods currently marketed in the U.S. at levels consistent with cGMP. These GRAS affirmed salts include ferric ammonium citrate, ferric chloride, ferric citrate, ferric phosphate, ferric pyrophosphate, ferric sulfate, ferrous ascorbate, ferrous carbonate, ferrous citrate, ferrous fumarate, ferrous gluconate, ferrous lactate, and ferrous sulfate with no limitation other than cGMP (21 CFR §184) (U.S. FDA, 2011). In accordance with the Fortification Policy outlined in the Nutritional Quality Guidelines for Foods set forth by the U.S. Food and Drug Administration (FDA) (21 CFR §104.20) (U.S. FDA, 2011), SFC will be added to food at levels at which there is a reasonable assurance that consumption of the food containing the added SFC will not result in an excessive intake of iron and will not be used to fortify fresh produce, meat, poultry, or fish products, sugars, or snack foods such as candies and carbonated beverages.

Based on the assumption that SFC would replace all dietary forms of iron in foods and supplements, the worst-case intake scenario of SFC was determined using the estimated intake of iron from foods and supplements by the Institute of Medicine (IOM) (IOM, 2001). The mean intake of iron ranged between 10.4 and 58.5 mg/person/day for children between 1 and 3 years of age and lactating women, respectively, whereas the 90<sup>th</sup> percentile intakes ranged from 17.6 and 112 mg/person/day for children between 1 and 3 years of age and lactating women, respectively. The potential intake of sodium from SFC was extrapolated from the estimated iron intake based on the sodium content of SFC (17.6%). The mean calculated intake of sodium from SFC for all age groups ranged between 17.5 and 35.7 mg/person/day, whereas the calculated 90<sup>th</sup> percentile intake of sodium for all age groups ranged between 29.8 and 58.4 mg/person/day, which are more than 74 and 64 times lower than the mean and 90<sup>th</sup> percentile dietary sodium intakes estimated by the IOM, respectively (IOM, 2004). The tolerable upper intake levels (ULs) for iron and sodium are 40 to 45 and 1,500 to 2,300 mg/ person/day, respectively (IOM, 2001, 2004). The worst-case 90<sup>th</sup> percentile intake scenarios of iron and

sodium are more than 1.3 and 30 times lower than the ULs of for iron and sodium, respectively (IOM, 2001, 2004).

SFC is a dietary source of organic iron and absorbed in the upper gastrointestinal tract, mainly the duodenum. Accordingly, SFC also provides sodium and citric acid/citrates as dietary sources based on the absorption mechanism of SFC. Sodium is almost entirely absorbed in the small intestine and excreted in the urine at concentrations equal to intake (IOM, 2004). Additionally, citric acid is well absorbed and is actively involved in the citric acid cycle (Nelson and Cox, 2000). Citric acid is a natural component in the diet and is regarded as a normal metabolite in the body and has a strong chelating action in the presence of iron (Helbock *et al.*, 1967; Forth *et al.*, 1968; Forth *et al.*, 1965).

The metabolic fate of iron was demonstrated in published studies, which is dependent on physiological need. Organic iron, as is the case for iron in SFC, is considered to be absorbed into the intestinal mucosa of the small intestine in two ways: the first as chelated iron consisting of iron and citric acid, and the second as dissociated ionic iron (Spiro *et al.*, 1967; Forth and Rummel, 1973; Fujita and Terato, 1973; Terato *et al.* 1973). During transportation from the mucosa to serosa in the small intestine, some unstable chelated iron may be dissociated through the presence of endogenous chelating agents and apo-ferritin. The ionic iron from the dissociated chelate is then transferred into the circulatory blood supply along with a small percentage of stable chelated iron. With regard to the stable chelated iron, the iron is considered to dissociate from the citric acid and bind to transferrin in blood. Transferrin then circulates throughout the body until iron is incorporated into certain proteins (*e.g.*, hemoglobin, myoglobin, and cytochromes) in the body that require iron for biological function. If iron is in excess, it is stored as ferritin and hemosiderin in the liver, bone marrow, and in reticuloendothelial cells. When cells are degraded, particularly erythrocytes, iron is largely recycled into new erythrocytes and thus, only small levels of iron are excreted primarily *via* the feces each day under normal conditions. Small amounts of iron also may be excreted *via* the urine, desquamated gastrointestinal cells, and bile. The metabolic fate of iron from SFC has been proven to be different from other sources of iron such as inorganic iron by Ariyoshi *et al.* (1987), Arizono *et al.* (1996), and Kobune *et al.* (2011). Moreover, Ariyoshi *et al.* (1987), Arizono *et al.* (1996), and Kobune *et al.* (2011) demonstrated that SFC is more bioavailable than ferrous sulfate, an already GRAS-affirmed iron fortification salt, suggesting that less SFC may be required to achieve adequate plasma concentrations of iron in comparison to other iron salts.

The safety of sodium, citric acid, and iron has been evaluated by scientific and regulatory agencies, including the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the IOM, and the FDA. The UL for sodium was established by the IOM to range from 1.5 to 2.2 g/day for children, and 2.3 g/day for adolescents and adults (IOM, 2004). JECFA determined an acceptable daily intake (ADI) of "not limited" for citric acid due to its well established role in oxidative metabolism and its lack of significant toxicological risk to human

health (JECFA, 1974). Additionally, JECFA established a provisional maximum tolerable daily intake (PMTDI) of 0.8 mg iron/kg body weight for all iron sources except iron oxides used in coloring agents in normal individuals, equivalent to a daily dose of 56 mg for a 70 kg individual (JECFA, 1983). Similarly, an UL for iron of 45 mg/person/day for adolescents and adults was derived by the IOM (2001).

Acute oral toxicity studies with SFC *via* the oral route in laboratory animals result in oral LD<sub>50</sub> values of 2,400 (male) or 4,100 (female) [equivalent to 240 or 410 mg iron(II)/kg body weight, respectively], 4,200 (male) or 4,000 (female) [approximately 420 or 400 mg iron(II)/kg body weight, respectively], and >5,000 [>500 mg iron(II)/kg body weight], in the mouse, rat, and dog, respectively (Sumikama *et al.*, 1985; Funahashi *et al.*, 1986). Reported adverse effects were consistent with effects observed for other dietary iron compounds, such as diarrhea, mild sedation, and discoloration in the liver. In repeat-dose studies conducted with rats and dogs, diarrhea and related effects also were observed at middle- to high-dose levels [*i.e.*, ≥3,000 and ≥150 mg SFC/kg body weight/day in rats and dogs, respectively, or ≥300 and ≥15 mg iron(II)/kg body weight/day, respectively] (Mitsuzono *et al.*, 1985; Funahashi *et al.*, 1986). In rats, a decrease in body weight was associated with decreases in food intake, as well as diarrhea, in groups administered greater or equal to 3,000 mg SFC/kg body weight/day, which were previously reported to be common effects associated with high intakes of iron (Mitsuzono *et al.*, 1985). The observed increases and decreases in relative and/or absolute organ weight (liver, kidney, thymus, and testes) were reported to be slight and were indicated to be associated with iron disposition in these organs. These observations of iron deposition, including hemosiderin deposits and brown pigmentation, were reported to be dose-dependent in some cases, in rats and dogs. Changes in urine pH were observed in rats and were reported to be associated with citrate. Based on the findings in rats, Mitsuzono *et al.* (1985) established a no-observed-adverse-effect level (NOAEL) for SFC of 300 mg/kg body weight/day; however, the authors determined a maximum safety level of 1,000 mg SFC/kg body weight/day based on the findings that the adverse effects were associated with the individual components of SFC. A NOAEL of 50 mg/kg body weight/day was established by Funahashi *et al.* (1986) for dogs based on the dose-dependent hemosiderin deposits in the liver. In another study, although inflammation, erosion, ulceration, and iron deposition were observed in the stomach of rats administered SFC or ferrous sulfate at 150 or 250 mg/kg body weight/day for 3 or 7 days, the effects were reported by the authors to be less severe in the SFC groups compared to the ferrous sulfate groups (Fujimori and Taki, 1987). In a reproductive toxicity study, no toxicologically relevant effects were observed among female rabbits administered up to 1,000 mg/kg body weight/day; however, a non-statistically significant increase in the incidence of spontaneous abortions was observed in the highest dose group (*i.e.*, 1,000 mg/kg body weight/day) in comparison to the control animal (Okada *et al.*, 1988). Although the authors attributed the spontaneous abortions to malnutrition as the females who aborted also had decreased food intakes and diffuse vacuolation of hepatocytes, the authors established a maternal NOAEL of 300 mg SFC/kg body weight/day. No biologically relevant adverse effects were observed in fetuses of mothers

administered SFC up to 1,000 mg/kg body weight/day. Therefore, a NOAEL for the offspring of 1,000 mg/kg body weight/day was established. Moreover, identified mutagenicity studies for SFC all indicated negative results (Mochida *et al.*, 1986). In comparison to toxicity studies conducted with other iron compounds (*e.g.*, ferrous sulfate) at high levels, the results observed in preclinical studies with SFC (*i.e.*, gastrointestinal disturbances) are similar in terms of the kind (type) of adverse reactions but lower in their incidence. Likewise, human studies following oral consumption of SFC (100 or 200 mg iron/day for up to 14 days, 100 or 200 mg iron/day for 5 to 37 weeks, or 2 mg iron/day for up to 60 days) did not result in serious adverse effects (Miyao *et al.*, 1984; Imamura and Kuramoto, 1987; Shirakura *et al.*, 1988). Furthermore, a study conducted by Imamura and Kuramoto (1987), demonstrated that SFC was without significant side effects following treatment for iron deficiency anemia. No hematological or biochemical side effects were reported in those patients that received SFC for a period of 10 weeks or longer.

The scientific evidence examined by the Panel demonstrates that under the conditions of intended use, SFC would not produce any adverse health effects.

## CONCLUSIONS

We, the Expert Panel, have, independently and collectively, critically evaluated the data and information summarized above and conclude that the intended uses of Sodium Ferrous Citrate (SFC), meeting appropriate food-grade specifications presented in the supporting dossier [**Documentation Supporting the Evaluation of Sodium Ferrous Citrate as Generally Recognized as Safe (GRAS) for Use as a Food Ingredient**] and produced consistent with current Good Manufacturing Practices (cGMP), are safe and suitable.

We further conclude that the intended uses of SFC, meeting appropriate food-grade specifications presented in the supporting dossier and produced consistent with cGMP, are Generally Recognized as Safe (GRAS) based on scientific procedures.

It is our opinion that other qualified experts would concur with these conclusions.

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Date

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31 October 2011  
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**Appendix B**

**Chemical and Microbial Analyses of 3-Non Consecutive Batches of  
Sodium Ferrous Citrate**

<b>Table B-1 Summary of the Chemical Product Analysis for 3 Lots of Sodium Ferrous Citrate</b>				
Specification Parameter	Specification	Manufacturing Lot		
		11A67S	11A70S	11B73S
Description	Green-white to greenish yellow powder. Odorless with a weak iron taste.	Conforms	Conforms	Conforms
Identification	Meets Requirements	Positive	Positive	Positive
Content (Assay, %)	10.0 to 11.0% of iron (Fe = 55.85)	10.4	10.4	10.3
Sulfate (%)	NMT 0.48% as SO <sub>4</sub>	≤0.48	≤0.48	≤0.48
Ferric salt	Meets requirements	Pass	Pass	Pass
Heavy Metals (ppm)	NMT 20 ppm as Pb	≤20	≤20	≤20
Lead (ppm)	NMT 1 ppm	≤1	≤1	≤1
Arsenic (ppm)	NMT 4 ppm as As <sub>2</sub> O <sub>3</sub>	≤4	≤4	≤4
Tartrate	Meets Requirements	Pass	Pass	Pass
Extraneous material (foreign matter)	NMT 3	Within limit	Within limit	Within limit

As<sub>2</sub>O<sub>3</sub>, arsenic oxide; Fe, iron; NMT, not more than; Pb, lead; SO<sub>4</sub>, sulfate

<b>Table B-2 Summary of the Microbiological Product Analysis for 3 Lots of Sodium Ferrous Citrate</b>				
Specification Parameter	Specification	Manufacturing Lot		
		65A70S	85A51S	05A52S
Total viable count				
Bacteria	NMT 100 CFU/g	0	0	0
Fungi	NMT 100 CFU/g	0	0	0
<i>Escherichia coli</i>	Negative	Negative	Negative	Negative
<i>Pseudomonas aeruginosa</i>	Negative	Negative	Negative	Negative
<i>Staphylococcus aureus</i>	Negative	Negative	Negative	Negative

CFU, colony forming units; NMT, not more than

<b>Table B-3 Summary of Methanol Analysis for 3 Non-Consecutive Batches of Sodium Ferrous Citrate</b>				
Parameter	Specification	Batch		
		64A28S	75A38S	84A70S
Methanol (µg/g)	NMT 50 µg/g	5.5	5.3	6.2

NMT, not more than



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MESSRS.

FEB, 7, 2011

# CERTIFICATE OF ANALYSIS

SANFEROL  
(SODIUM FERROUS CITRATE)

LOT NUMBER 11A67S

DESCRIPTION	GOOD /
IDENTIFICATION	POSITIVE /
SULFATE (not more than 0.48%)	WITHIN LIMIT /
FERRIC SALT	Pass Test /
HEAVY METALS (not more than 20 μg/g)	WITHIN LIMIT /
ARSENIC (not more than 4.0 μg/g)	WITHIN LIMIT /
TARTRATE	Pass Test /
ASSAY (Fe)	10.4 % /
EXTRANEIOUS MATERIAL	WITHIN LIMIT /

TESTS AND STANDARDS SANNOVA STD

EVALUATION PASSED /

EXPIRY DATE NOV, 30, 2012

MFG. DATE DEC, 15, 2010

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OCT. 19, 2011

CERTIFICATE OF ANALYSIS

SANFEROL  
(SODIUM FERROUS CITRATE)

LOT NUMBER 11A70S

DESCRIPTION	GOOD
IDENTIFICATION	POSITIVE
SULFATE (not more than 0.48%) FERRIC SALT	WITHIN LIMIT Pass Test
HEAVY METALS (not more than 20 μg/g)	WITHIN LIMIT
ARSENIC (not more than 4.0 μg/g)	WITHIN LIMIT
TARTRATE	Pass Test
ASSAY (Fe)	10.4 %
EXTRANEIOUS MATERIAL	WITHIN LIMIT

TESTS AND STANDARDS SANNOVA STD

EVALUATION PASSED

EXPIRY DATE NOV. 30, 2012

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MANAGER OF QUALITY CONTROL LAB.

T. Okamoto



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サンノーバ株式会社

群馬県太田市世良田町3038-2

Sannova Co., Ltd

3038-2, Serada-cho, Ota-shi  
Gunma 370-0426, Japan

MESSRS.

FEB, 7, 2011

# CERTIFICATE OF ANALYSIS

SANFEROL  
(SODIUM FERROUS CITRATE)

LOT NUMBER 11B73S

DESCRIPTION	GOOD	/
IDENTIFICATION	POSITIVE	/
SULFATE (not more than 0.48%)	WITHIN LIMIT	/
FERRIC SALT	Pass Test	/
HEAVY METALS (not more than 20 $\mu$ g/g)	WITHIN LIMIT	/
ARSENIC (not more than 4.0 $\mu$ g/g)	WITHIN LIMIT	/
TARTRATE	Pass Test	/
ASSAY (Fe)	10.4 %	/
EXTRANEIOUS MATERIAL	WITHIN LIMIT	/

TESTS AND STANDARDS SANNOVA STD

EVALUATION PASSED /

EXPIRY DATE NOV, 30, 2012

MFG. DATE DEC, 15, 2010

(b) (6)

IKUO OYA  
MANAGER OF QUALITY CONTROL LAB.



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CERTIFICATE OF ANALYSIS

Client: Eisai Food & Chemical Co., Ltd.  
2-13-10 Nihonbashi, Chuo-ku, Tokyo 103-0027, Japan

Sample name: Product: Sanferol (Sodium Ferrous Citrate) Lot No: 06A49S

Received date: July 11, 2011

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

Test Result(s)

Test Item	Result	QL	N	M
Lead	Not more than 1 ppm	-----	1	

QL: Quantitation limit N: Notes M: Method

Notes

1:FCC VII General Tests and Assays "LEAD LIMIT TEST" APDC Extraction Method.



(b) (6)

Noriko Imaizumi  
Principal Investigator

Date

Jul. 26, 2011

**CERTIFICATE OF ANALYSIS**

Client: Eisai Food & Chemical Co., Ltd.  
2-13-10 Nihonbashi, Chuo-ku, Tokyo 103-0027, Japan

Sample name: Product: Sanferol (Sodium Ferrous Citrate) Lot No: 09A21S

Received date: July 11, 2011

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

**Test Result(s)**

Test Item	Result	QL	N	M
Lead	Not more than 1 ppm	.....	1	

QL: Quantitation limit N: Notes M: Method

**Notes**

1:FCC VII General Tests and Assays "LEAD LIMIT TEST" APDC Extraction Method.



(b) (6)

Noriko Imaizumi  
Principal Investigator

Date

Jul. 26, 2011

## CERTIFICATE OF ANALYSIS

Client: Eisai Food & Chemical Co., Ltd.  
2-13-10 Nihonbashi, Chuo-ku, Tokyo 103-0027, Japan

Sample name: Product: Sanferol (Sodium Ferrous Citrate) Lot No: 11A53S

Received date: July 11, 2011

This is to certify that the following result(s) have been obtained from our analysis on the above-mentioned sample(s) submitted by the client.

## Test Result(s)

Test Item	Result	QL	N	M
Lead	Not more than 1 ppm	-----	1	

QL: Quantitation limit N: Notes M: Method

## Notes

1:FCC VII General Tests and Assays "LEAD LIMIT TEST" APDC Extraction Method.



(b) (6)

Noriko Imaizumi  
Principal Investigator

Jul. 26, 2011  
Date



Sannova Co., Ltd.

Head Office:  
30-21 Sanno-cho, Otsubo  
Gumma 370-0426 JAPAN

発行日  
2011. 9. 15

## サンフェロールの残留溶媒(メタノール)試験結果報告書

試験項目：サンフェロール残留溶媒 (メタノール)

規格値：50  $\mu$ g/g以下

試験結果：

製品ロット	試験結果
64A28S	5.5 $\mu$ g/g
75A38S	5.3 $\mu$ g/g
84A70S	6.2 $\mu$ g/g

(b) (6)

Ikuo Oya, Director  
Quality Control

Sannova Co., Ltd.

(b) (6)

Hiroyuki Fujimori, Executive Director  
Corporate Regulatory Compliance and QA Dept.

Sannova Co., Ltd.

# Microbial Limit Tests

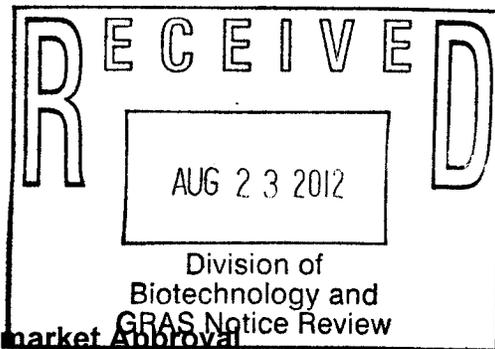
Eisai Food and Chemical Co., Ltd.  
Sannova Co., Ltd.

**Product: SANFEROL (Sodium Ferrous Cotrate)**

Specifications		Lot. 65A70S	
		Measurement	Judgment
Fungus	Not more than 100/g	0 (Not more than 100/g)	Conformed
Coliform population	Negative	Negative	Conformed
<i>Staphylococcus aureus</i>	Negative	Negative	Conformed
<i>Pseudomonas aeruginosa</i>	Negative	Negative	Conformed
Bacteria	Not more than 100/g	0 (Not more than 100/g)	Conformed

Specifications		Lot. 85A51S	
		Measurement	Judgment
Fungus	Not more than 100/g	0 (Not more than 100/g)	Conformed
Coliform population	Negative	Negative	Conformed
<i>Staphylococcus aureus</i>	Negative	Negative	Conformed
<i>Pseudomonas aeruginosa</i>	Negative	Negative	Conformed
Bacteria	Not more than 100/g	0 (Not more than 100/g)	Conformed

Specifications		Lot. 05A52S	
		Measurement	Judgment
Fungus	Not more than 100/g	0 (Not more than 100/g)	Conformed
Coliform population	Negative	Negative	Conformed
<i>Staphylococcus aureus</i>	Negative	Negative	Conformed
<i>Pseudomonas aeruginosa</i>	Negative	Negative	Conformed
Bacteria	Not more than 100/g	0 (Not more than 100/g)	Conformed



**I GRAS EXEMPTION CLAIM**

**A. Claim of Exemption From the Requirement for Premarket Approval Pursuant to Proposed 21 CFR §170.36(c)(1) [62 FR 18938 (17 April 1997)]**

Eisai Food & Chemical Co., Ltd. has determined sodium ferrous citrate to be Generally Recognized as Safe (GRAS), consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act*. This determination is based on scientific procedures as described in the following sections, under the conditions of its intended use in food, among experts qualified by scientific training and expertise. Therefore, the use of sodium ferrous citrate in food as described below is exempt from the requirement of premarket approval.

Signed,

(b) (6)



Minoru Tanaka  
Eisai Food & Chemical Co., Ltd.  
5<sup>th</sup> Floor Nihonbashi Sunrise Bldg.  
2-13-10 Nihonbashi Chuo-ku Tokyo  
103-0027 Japan

Date July 30, 2012

**B. Name and Address of Notifier**

Minoru Tanaka  
Eisai Food & Chemical Co., Ltd.  
5<sup>th</sup> Floor Nihonbashi Sunrise Bldg.  
2-13-10 Nihonbashi Chuo-ku Tokyo  
103-0027 Japan  
Telephone: +81-3-3548-3560  
Facsimile: +81-3-3273-2084  
Email: [m-tanaka@eisai-fc.co.jp](mailto:m-tanaka@eisai-fc.co.jp)

**C. Common Name of the Notified Substance**

Sodium ferrous citrate

**Ramos-Valle, Moraima**

---

**From:** Rebecca Rogerson Intertek [rebecca.rogerson@intertek.com]  
**Sent:** Thursday, August 23, 2012 8:46 AM  
**To:** Ramos-Valle, Moraima  
**Cc:** Shepherd, Lillian; Ashley Roberts Intertek  
**Subject:** RE: GRAS submission sodium ferrous citrate  
**Attachments:** GRAS Signature page\_revised 22Aug2012.pdf

Dear Ms. Ramos-Valle,

On behalf of Dr. Roberts, I am sending the amended page 3 making the requested change to include the company name. I hope that the revised page 3 will overcome the technicality and enable the notification to be filed?

I look forward to receiving your reply

Kind Regards  
Rebecca

---

**Rebecca Rogerson, B.Sc**  
**Scientific & Regulatory Consultant**  
**Food and Nutrition Group**  
**Intertek Cantox**  
2233 Argentia Rd, Suite 308, Mississauga, ON, Canada, L5N 2X7  
Tel: (905) 286-4180 | Fax: (905) 542-1011 | Email: [rebecca.rogerson@intertek.com](mailto:rebecca.rogerson@intertek.com)  
Skype: rebecca.rogerson.intertek  
<http://www.intertek.com/cantox/>

---

**From:** Ramos-Valle, Moraima [Moraima.Ramos-Valle@fda.hhs.gov]  
**Sent:** August 22, 2012 7:44 PM  
**To:** Ashley Roberts Intertek  
**Cc:** Shepherd, Lillian  
**Subject:** GRAS submission sodium ferrous citrate

Dear Dr. Roberts,

This message is to inform you of our receipt of Eisai Food & Chemical Co., Ltd. GRAS submission for sodium ferrous citrate dated July 30, 2012. As we review your submission for filing, we noted that the GRAS exemption claim section needs to be amended. On page 3 of your submission, section A, please amend this statement to say that Eisai Food & Chemical Co., Ltd. has determined that .....(the ingredient) is GRAS ...

It is very important that this statement clearly state the name of the company and that the company is the one making the GRAS determination for that ingredient, since the notifier is the one responsible for it.

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8/23/2012

If you have any questions, please feel free to contact me.

**Moraima J. Ramos Valle, M.S.**

Consumer Safety Officer

Division of Biotechnology and GRAS Notice Review

Food and Drug Administration

Phone: 240-402-1248

Email: [Moraima.Ramos-Valle@fda.hhs.gov](mailto:Moraima.Ramos-Valle@fda.hhs.gov)

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Pages 000066-000321 have been removed in accordance with copyright laws. The removed references are:

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SUBMISSION END