GRAS Determination of the Complexation Products of Iron with Sodium Citrate for Use in Salt Used in Foods

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GRAS Determination of the Complexation Products of Iron with Sodium Citrate for Use in Salt Used in Foods

SUBMITTED BY:

Cargill, Inc.
15407 McGinty Road West
Wayzata, MN 55391

SUBMITTED TO:

U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
HFS-200
5100 Paint Branch Parkway
College Park MD 20740-3835

CONTACT FOR TECHNICAL OR OTHER INFORMATION

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Acronyms

ADME	absorption, distribution, metabolism, and excretion
CAS	Chemical Abstracts Service
CFR	Code of Federal Regulations
cGMP	current Good Manufacturing Practice
CKD	chronic kidney disease
EDTA	sodium iron ethylenediaminetetraacetic acid
FAO	Food and Agriculture Organization of the United Nations
FDA	U.S. Food and Drug Administration
GRAS	Generally Recognized as Safe
GRN	GRAS Notification
IOM	Institute of Medicine
JECFA	Joint FAO/WHO Expert Committee on Food Additives
NHANES	National Health and Nutrition Examination Survey
NOAEL	no-observed-adverse-effect level
PMTDI	provisional maximum tolerable daily intake
RDA	recommended daily allowance
SCOGS	Select Committee on GRAS Substances
STP	standard temperature and pressure
UL	tolerable upper intake limit
U.S.C.	United States Code
USDA	United States Department of Agriculture
WHO	World Health Organization

§ 170.225 Part 1, GRAS Notice: Signed Statements and Certification

(1) GRAS Notice Submission

Cargill, Incorporated (Cargill), through its agent, ToxStrategies, Inc., hereby notifies the U.S. Food and Drug Administration (FDA) of the submission of a Generally Recognized as Safe (GRAS) notice for the use of the complexation products of iron with sodium citrate for use in salt used in human food, in accordance with Subpart E of 21 CFR § 170.

(2) Name and Address

Cargill, Incorporated
15407 McGinty Road West
Wayzata, MN 55391

(3) Name of Notified Substance

The name of the substance that is the subject of this Generally Recognized as Safe (GRAS) notification is the complexation products of iron with sodium citrate (hereafter referred to as iron citrate).

(4) Intended Use in Food

The iron citrate is intended for use as an anti-caking agent in salt at a use level of up to 135 ppm (or 30 ppm calculated as iron).

(5) Statutory Basis for GRAS Determination

Cargill, through its agent ToxStrategies, Inc., confirms that the iron citrate ingredient, meeting the specifications described herein, has been determined to be GRAS through scientific procedures in accordance with 21 CFR § 170.30(a) and (b).

(6) Premarket Approval Statement

Cargill further asserts that the use of iron citrate in salt used in foods, as described below, is exempt from the pre-market approval requirements of the Federal Food, Drug, and Cosmetic Act, based on a conclusion that the notified substance is GRAS under the conditions of its intended use.

(7) Availability of Information

The data and information that serve as the basis for this GRAS determination, as well as any information that has become available since the GRAS determination, will be sent to

the U.S. Food and Drug Administration (FDA) on request, or are available for the FDA's review and copying during customary business hours from ToxStrategies, Inc., Naperville, Illinois.

(8) Data and Information Confidentiality Statement

None of the data and information items in the GRAS notice are exempt from disclosure under the Freedom of Information Act, 5 U.S.C. 552.

(9) GRAS Notice Certification

To the best of our knowledge, this GRAS determination is a complete, representative, and balanced document. Cargill is not aware of any information that would be inconsistent with a finding that the proposed uses and use levels of iron citrate in salt used in food, meeting the appropriate specifications described herein, and used according to current Good Manufacturing Practices (cGMP), is GRAS. Recent reviews of the scientific literature revealed no potential adverse health concerns.

(10) Name/Position of Notifier

_____ _____

Donald F. Schmitt, M.P.H. Date
Senior Managing Scientist
ToxStrategies, Inc.
Agent for Cargill, Inc.

(11) FSIS Statement

The iron citrate product will not be used in products under the jurisdiction of the U.S. Department of Agriculture (USDA).

§ 170.230 Part 2, Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

Identity

The subject of this GRAS determination is the complexation products of iron with sodium citrate (referred to in this document as iron citrate). It is a dark green/red aqueous solution intended for use as an anti-caking agent in salt. Anticaking agents function either by adsorbing excess moisture, or by coating particles and making them water and oil repellent. In the U.S., calcium silicate (CaSiO3), for example, is a common anti-caking agent added to table salt as it absorbs both water and oil. In Europe, sodium ferrocyanide and potassium ferrocyanide are more common anti-caking agents used in table salt.

Common or Chemical Names

The common name of the proposed anti-caking agent is iron citrate. Its chemical name is best described as the complexation products of iron with sodium citrate. The iron source can be any food-grade ferrous iron salt such as iron (II) sulfate, iron (II) chloride, or a mixture of these iron salts. A Chemical Abstracts Service (CAS) number has not been assigned.

Chemical Formula

The chemical formula of the complexation products of iron with sodium citrate is $FeC_6H_5O_6Na$ (nominal) and the molecular weight is 252.95 (nominal).

Physical Properties

The iron citrate product is a dark green/red aqueous solution comprising at least 22% by weight of the complexation products of iron with citrate, with a pH between 5 and 10. It contains not less than 3.5% total iron (calculated as the element on a dry basis) and not less than 11.5% citrate (calculated as the trisodium salt on a dry basis). Whether in a ferrous or ferric state, the iron citrate ingredient retains its anti-caking effect.

Manufacturing Process

Iron citrate is prepared from a ferrous iron salt (1.0 molar equivalent Fe) and food grade sodium citrate (1.0 molar equivalent), with sodium hydroxide (0.5–1.2 molar equivalents) added for pH adjustment. The iron source can be any food-grade ferrous iron salt such as iron (II) sulfate, iron (II) chloride, or a mixture of these iron salts. The counterions from the iron salt are inconsequential to the function of the iron citrate solution and are purely spectator ions and do not disrupt the iron-citrate complex as long as they are non-chelating. Sodium hydroxide is included in the solution for pH adjustment to the desired range of 5–10. Mixing the components in water at standard temperature and pressure (STP) will produce the desired dark green/red solution. The solution can then be

transferred to appropriate liquid storage containers, with any necessary physical treatment (particulate filtration, cooling). Sodium hydroxide (CAS No. 1310-73-2; see CFR § 184.1763) is the only processing aid used in the production of the iron citrate product and is commonly used in food ingredient manufacturing processes. Ultimately, the solution is applied by spraying directly onto the salt.

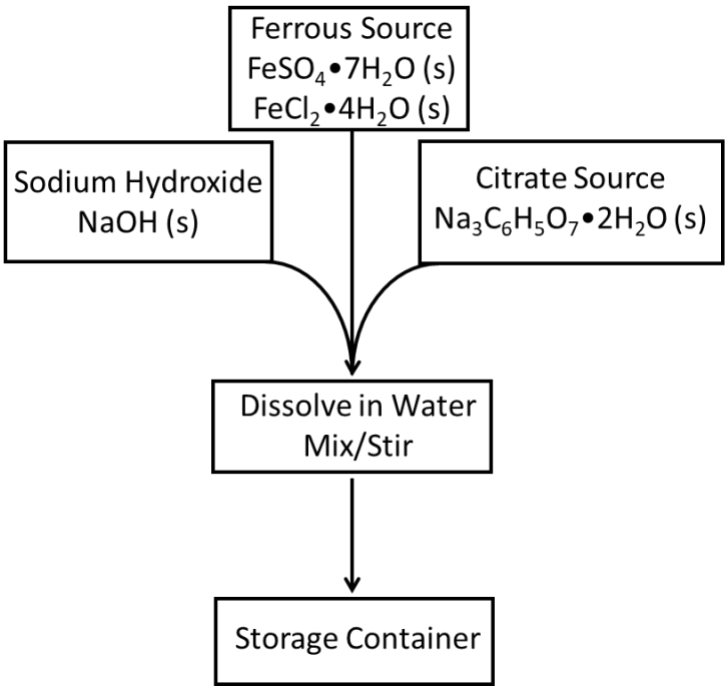

Figure 1. Manufacturing process flow diagram: Complexation products of iron with sodium citrate

Product Specifications

The proposed food-grade specifications for Cargill's iron citrate product and analytical data from three non-consecutive lots are presented in Tables 1 and 2, and the mass balance of the iron citrate product in Table 3.

Table 1. Specifications for iron citrate complexation product (22% w/w aqueous solution)

Parameter	Specification	Assay/Analytical Method
Appearance	Dark aqueous solution	
Assay-total iron (%)	>3.5	ICP-OES; spectrophotometry
Assay-citrate (%)	>11.5	Ion chromatography
pH	5.0 – 10.0	pH meter
Density (g/mL)	>1.20	Gravimetric
Identification	Passes test for iron and citrate	FCC; Appendix IIIA
Water (%)	≤78	Gravimetric
Chloride	Not more than 2.2:1 (molar basis) vs Fe	Ion chromatography
Sulfate	Not more than 1.2:1 (molar basis) vs. Fe	Ion chromatography
Sodium	Not more than 5.6:1 (molar basis) vs. Fe	ICP-OES
Arsenic (ppm)	≤1	ISO 11885
Lead (ppm)	≤3	ISO 11885
Mercury (ppm)	≤1	ISO 11885
Cadmium (ppm)	≤1	ISO 11885

ICP-OES – Inductively coupled plasma - optical emission spectrometry; FCC – Food Chemicals Codex; ISO – International Organization for Standardization

Table 2. Analytical results for four lots of iron citrate (22% w/w aqueous solution)

Parameter		Specification	Batch A	Batch B	Batch C	Batch D
Definition						
Assay	Total Iron	Not less than 3.5%, calculated as element on dry basis	4.3%	4.2%	4.3%	4.2%
	Citrate	Not less than 11.5%, calculated as the Citrate (3-) tri-anion on dry basis	13.4%	14.2%	12.9%	13.2%
Description		Dark green/red aqueous solution comprising at least 22% by weight the complexation products of iron and citrate, with a pH between 5 and 10	Pass pH 7.0	Pass pH 9.1	Pass pH 5.6	Pass pH 8.5
Density		>1.20 g/mL	1.26	1.27	1.21	1.22
Identification		Passes tests for iron and citrate	Pass	Pass	Pass	Pass

Parameter	Specification	Batch A	Batch B	Batch C	Batch D
Purity					
Water	Not more than 78%	68%	68%	71%	69%
Chloride	Not more than 2.2:1 (molar basis) vs Fe	0.01:1	0.00:1	1.50:1	1.52:1
Sulfate	Not more than 1.2:1 (molar basis) vs Fe	0.80:1	0.81:1	0.00:1	0.00:1
Sodium	Not more than 5.6:1 (molar basis) vs Fe	3.62:1	3.93:1	3.56:1	3.86:1
Arsenic	Not more than 1 ppm	ND	ND	ND	ND
Lead	Not more than 3 ppm	2.70*	2.56	2.63	2.55
Mercury	Not more than 1 ppm	ND	ND	ND	ND
Cadmium	Not more than 1 ppm	ND	ND	ND	ND

ND – not detected (detection limit > 0.5 ppm)

*at a dietary intake of approximately 1 mg iron citrate per day (see Dietary Exposure section), the lead intake from a concentration of 3.0 mg/kg in iron citrate represents an intake of lead of approximately 3.0 nanograms/day.

Table 3. Relative proportion of each component of the complexation product of iron with sodium citrate (22% w/w aqueous solution)

	Calculated Concentration			
Component (%)	**Batch A**	**Batch B**	**Batch C**	**Batch D**
Total iron	4.3	4.2	4.3	4.2
Citrate	13.4	14.2	12.9	13.2
Chloride	0.0	0.0	5.3	5.4
Sulfate	7.7	7.8	0.0	0.0
Sodium	6.4	6.9	6.3	6.7
Water	68	68	71	69
Total (mass balance)	99.8	101.1	99.8	98.5

*Batches A and B produced with ferrous sulfate; batches C and D with ferrous chloride.

In summary, the analytical results demonstrate that the iron citrate product is consistently manufactured to meet the established specifications and does not contain unacceptable levels of contaminants. It should be noted that Cargill is actively working toward further reducing the lead concentration in the iron citrate anti-caking agent despite the very low estimated exposure from its use in salt (i.e., 3.0 nanograms/day; below 3 ug/day for children-FDA 2018 new maximum daily intake level). The analytical results also confirm the absence of impurities and contaminants (e.g., heavy metals).

Stability Data

The degradation of carboxylic acids in the presence of trace levels of iron is well established to occur by photochemistry and Fenton chemistry (Abrahamson et al., 1994; Clark et al., 2007). Similar degradation processes would be anticipated to occur in the complexation products of iron with sodium citrate, if exposed to light or chemical oxidants. Cargill has determined the appropriate preparation and storage conditions and has also established suitable indicators of degradation (see Table 4). It has been observed that solutions stored with no special precautions on the benchtop (room temperature, reasonably capped) do not show any decrease in stability and anti-caking effectiveness after 4 months.

Table 4. Indicators of iron citrate product degradation

Factor	Potential Role as Indicator of Stability
Precipitation	Formation of a precipitate could indicate loss of active iron and citrate species from the solution. (e.g., Fe/Citrates, iron hydroxide/oxide species, etc.).
Loss of color	Loss of the characteristic dark color of the iron citrate solution may indicate photochemical degradation of the active iron citrate complex, resulting in decreased effectiveness.
pH	Changes in pH may indicate changes in iron citrate complex speciation and result in reduced effectiveness.
Content of constituents	Specific concentration of individual constituents may indicate degradation or precipitation from solution.

No decrease in stability and anti-caking effectiveness is observed for iron citrate solutions stored under "normal" conditions (2–20 °C, in a capped container) for up to 4 months, the longest time tested. Solutions of the complexation products of iron with sodium citrate stored in direct sunlight at room temperature were not stable (i.e., precipitation, loss of color) and decomposition occurred within days. In addition, some solutions of the complexation products of iron with sodium citrate that are stored in uncapped containers, exposed to atmosphere, do exhibit some evidence of degradation (i.e., precipitation) within days. No difference in stability was observed for iron citrate solutions stored in a sealed container at temperatures up to 32 °C. Therefore, iron citrate solutions will be stored in a closed container at room temperatures between 2 – 20 °C and kept out of direct sunlight or stored in amber bottles. The stability testing report can be found in Appendix B. In addition, a warehouse study is ongoing (> 30 days currently) wherein salt treated with iron citrate is stored under normal warehouse conditions and evaluated for anti-caking effectiveness against salt treated with tricalcium phosphate (TCP). It is expected that the iron citrate anticaking agent will be a stable and effective anti-caking agent in salt for a minimum of one year, similar to salt with TCP.

§ 170.235 Part 3, Dietary Exposure

Cargill intends to market the iron citrate ingredient (i.e., complexation product of iron and sodium citrate) as an anti-caking agent in salt at a use level ≤135 ppm (or 30 ppm calculated as iron). The iron citrate product will be used as an alternative to, not in addition to, other anti-caking agents used in salt (i.e., sodium ferrocyanide decahydrate, 21 CFR §172.490; iron complex of tartaric acid, GRN 532; calcium silicate, 21 CFR § 172.410; tricalcium phosphate, 21 CFR § 182.8217).

The Recommended Dietary Allowance (RDA) for all age groups of men and postmenopausal women is 8 mg/day; the RDA for premenopausal women is 18 mg/day. The median dietary intake of iron is approximately 16 to 18 mg/day for men and 12 mg/day for women. The Tolerable Upper Intake Level (UL) for adults is 45 mg/day of iron, a level based on gastrointestinal distress as an adverse effect (IOM, 2001). This is discussed in greater detail in the Safety section below.

Wallace et al. (2019) reported on the current sodium intakes in the United States. Employing NHANES 2013–2016 data, Americans (aged >1 year) consumed approximately 3361 mg sodium/day on average. Mean daily sodium intake from foods and beverages among the U.S. population was 2906 mg/day for children (aged 1–18 years) and 3499 mg/day for adults (aged ≥19 years).

Mean estimates of salt intakes have been calculated based on reported sodium consumption and were used to determine the potential exposure of the general population to the complexation product of iron and sodium citrate. The calculated intakes assumed that all sodium was consumed as salt by the general population, and that all salt contained the anti-caking agent at a maximum level of 135 ppm (or 30 ppm calculated as iron).

Sodium makes up 40% of salt (sodium chloride); therefore, the actual intake of salt for the above age groups would be as follows:

- Age >1 year; 3361 mg sodium = 8402 mg salt
- Ages 1-3 years; 2063 mg sodium = 5156 mg salt
- Ages 1–18 years; 2906 mg sodium = 7265 mg salt
- Ages ≥19 years; 3499 mg sodium = 8748 mg salt

Based on the maximum use level of 135 ppm (mg/kg salt) of the proposed anti-caking agent (equivalent to 30 ppm iron or 30 mg iron/kg salt), the potential daily exposure to iron citrate and iron are as follows:

- Age >1 year; 1.13 mg iron citrate/day; 0.25 mg iron/day
- Ages 1-3 years; 0.70 mg iron citrate/day; 0.16 mg iron/day
- Ages 1–18 years; 0.98 mg iron citrate/day; 0.22 mg iron/day

- Ages ≥19 years; 1.18 mg iron citrate/day; 0.26 mg iron/day

As described above, these estimates can be considered over-estimates of the true exposure by the general U.S. population. It is unlikely that the iron citrate ingredient will replace all other anti-caking agents currently added to salt. In addition, it is unlikely that a consumer would consume only salt with added iron citrate.

§ 170.240 Part 4, Self-Limiting Levels of Use

The use of the proposed iron citrate product in salt is considered self-limiting for technological reasons, such as product texture and/or flavor profile. There is also no advantage to using higher levels than those required to achieve the desired anti-caking effect in salt.

§ 170.245 Part 5, Experience Based on Common Use in Food

The statutory basis for our conclusion of GRAS status in the notice is based on scientific procedures and not common use in food.

§ 170.250 Part 6, GRAS Narrative

History of Use and Regulatory Approval

Ferric citrate is considered GRAS for use in food as a nutrient supplement, as well as in infant formula, as stated in 21 CFR §184.1298:

> **21 CFR §184.1298 Ferric citrate.**
>
> (a) Ferric citrate (iron (III) citrate, C6H5FeO7, CAS Reg. No. 2338-05-8) is prepared from reaction of citric acid with ferric hydroxide. It is a compound of indefinite ratio of citric acid and iron.
>
> (b) The ingredient must be of a purity suitable for its intended use.
>
> (c) In accordance with § 184.1(b)(1), the ingredient is used in food as a nutrient supplement as defined in § 170.3(o)(20) of this chapter, with no limitation other than current good manufacturing practice. The ingredient may also be used in infant formula in accordance with section 412(g) of the Federal Food, Drug, and Cosmetic Act (the act) (21 U.S.C. 350a(g)) or with regulations promulgated under section 412(a)(2) of the act (21 U.S.C. 350a(a)(2)).
>
> (d) Prior sanctions for this ingredient different from the uses established in this section do not exist or have been waived.

Ferrous citrate is considered GRAS for use in food as a nutrient supplement, as well as in infant formula, as stated in 21 CFR §184.1307c:

> **21 CFR §184.1307c Ferrous citrate.**
>
> (a) ferrous citrate (iron (II) citrate, (C6H6FeO7), CAS Reg. No. 23383-11-1) is a slightly colored powder or white crystals. It is prepared from the reaction of sodium citrate with ferrous sulfate or by direct action of citric acid on iron filings.
>
> (b) The ingredient must be of a purity suitable for its intended use.
>
> (c) In accordance with § 184.1(b)(1) the ingredient is used in food as a nutrient supplement as defined in § 170.3(o)(20) of this chapter, with no limitation other than current good manufacturing practice. The ingredient may also be used in infant formula in accordance with section 412(g) of the Federal Food, Drug, and Cosmetic Act (the act) (21 U.S.C. 350a(g)) or with regulations promulgated under section 412(a)(2) of the act (21 U.S.C. 350a(a)(2)).
>
> (d) Prior sanctions for this ingredient different from the uses established in this section do not exist or have been waived.

Numerous iron compounds (e.g., reduced, electrolytic, and carbonyl iron, ferrous ascorbate, ferrous carbonate, ferrous citrate, ferrous fumarate, ferrous gluconate, ferrous lactate, ferrous sulfate, ferric ammonium citrate, ferric citrate, ferric phosphate, or ferric pyrophosphate) are recognized as GRAS for their intended uses in foods (SCOGS, 1980).

Moreover, sodium iron ethylenediaminetetraacetic acid (EDTA) (GRAS Notice No. GRN 000152 and GRN 000178), ferrous ammonium phosphate (GRAS Notice No. GRN 000271), sodium ferrous citrate (GRAS Notice No. GRN 000441), a complexation product of sodium tartrates with iron (III) chloride (GRAS Notice No. GRN 000532), and iron milk proteinate (GRAS Notice No. GRN 000959) are considered GRAS for use as dietary iron sources for fortification purposes in selected foods or as an anti-caking agent in salt (GRN 532). The proposed use of iron citrate as an anti-caking agent in salt is not a significant dietary source of iron.

Safety

Introduction

The safety assessment of iron citrate as an anti-caking agent under its intended conditions of use and estimated intakes is primarily based on information pertaining to the safety of the individual components, iron and citrate. Ferric citrate is the iron (III) salt of citric acid and is made from a reaction of citric acid with ferric hydroxide. Ferrous citrate is the iron (II) salt of citric acid and is made from the reaction of sodium citrate with ferrous sulfate or by direct action of citric acid on iron filings. Iron is an essential trace mineral in the human diet and is found naturally in several foods, including legumes, green vegetables, meat, and seafood. Foods, such as breads and cereals, may also be fortified with iron and can also be available as a dietary supplement (Ems et al., 2021). Several iron (III) and (II) salts and ingredients are listed as direct food substances affirmed as GRAS in Title 21 CFR (U.S. FDA, 2021), with no limitation on their use other than cGMP. This reflects the long history of use of iron sources as food ingredients.

In 2001, the Food and Nutrition Board of the Institute of Medicine (IOM) reviewed the available literature on iron to establish recommended daily allowances (RDAs) and tolerable upper intake limits (ULs) for different populations in the United States (IOM, 2001). The RDA for iron is 8 mg/day for all groups of men and postmenopausal women and 18 mg/day for premenopausal women. A UL of 40 mg/day for children (1–13 years old) and 45 mg/day adults (>14 years old) was established. In 1983, JECFA evaluated dietary iron and established a provisional maximum tolerable daily intake (PMTDI) of 0.8 mg/kg-bw/day, or 56 mg/day based on a 70-kg adult. The evaluation included all sources of iron except iron oxide from coloring agents, supplemental iron taken during pregnancy and lactation, and supplemental iron for specific clinical requirements (JECFA 1983).

The calculated iron exposure of an individual from use of the ferric citrate product as an anti-caking agent in salt (0.26 mg/person/day) is low, and the contribution to background dietary intakes of iron would be negligible, because the mean iron intake in the United States is approximately 16 to 18 mg/day for men and 12 mg/day for women; 90th percentile intake was approximately 25 to 31 mg/day for men and 18 to 20 mg/day for women (IOM, 2001). Therefore, there is no potential for exceeding the UL based on the intended use of iron citrate as an anti-caking agent in salt, and for this reason, the risk of adverse effects from this constituent is minimal.

Citrates, or citric acid, is readily absorbed by the stomach, and about 2 kg of citric acid is formed and metabolized as an energy source every day in humans. It is involved in oxidative metabolism, because it is an intermediate in the citric acid (Krebs) cycle, breaking down pyruvate formed from glucose through glycolysis. It is freely filterable at the glomerulus of the kidney, with 65%–90% reabsorbed and 10%–35% excreted through the urine. The normal blood citrate level in humans is approximately 25 mg/L (Kuether and Smith, 1940; Fiume et al., 2014). Citric acid is also affirmed as GRAS for use in food in 21 CFR §184.1033. JECFA also evaluated citric acid in 1973 and concluded that it is not a significant toxicological risk to human health and set an acceptable daily intake (ADI) of "not limited" (JECFA, 1974).

For the present GRAS determination of iron citrate, comprehensive literature searches were performed pertinent to its safety and intended use and use level. Literature searches have been performed to identify available safety data through August 2021. These included searching sources of information such as publicly available assessments, databases, and reviews from organizations that include EFSA, the Joint FAO/WHO Expert Committee on Food Additives (JECFA), U.S. FDA, and the World Health Organization (WHO); as well as general internet searching and searching databases such as Embase, Medline, Toxline, and PubMed.

Safety Data

Below are summaries of toxicity studies specific to iron, ferric citrate, and ferrous citrate. The proposed ingredient, complexation products of iron with sodium citrate is a mixture and can contain both ferrous and ferric forms of iron citrate and thus the study summaries presented below are directly relevant to an evaluation of its safety. The studies include short-term, subchronic, and chronic/carcinogenicity studies, as well as mutagenicity/ genotoxicity studies.

Animal Studies

Absorption, Distribution, Metabolism, and Excretion (ADME)

Floege et al. (2020) examined iron uptake and accumulation in a 90-day oral gavage study in healthy Sprague Dawley rats. Rats (n=40/sex) 7–9 weeks old were divided into four groups (n=10/sex/group): (1) vehicle-only control, (2) ferrous sulphate heptahydrate in vehicle, (3) sucroferric oxyhydroxide in vehicle, and (4) ferric citrate. The specific group of interest in this study is (4) ferric citrate, which was treated at 50 mg Fe/kg-bw (equivalent to 10 mg Fe/mL in 1.5% methylcellulose vehicle). All surviving animals were euthanized and perfused. Samples of the liver and spleen were collected to quantify iron, and blood was collected to measure serum iron. The liver, spleen, kidney, heart, brain, and sternum (with bone marrow) were collected for histopathology. Statistically significant increases in the ferric citrate group were observed in the following: liver iron (two-fold), with females consistently being higher than male rats; total iron spleen content in males only; and mean serum iron when compared to control. Histological evaluation with Prussian blue staining indicated a higher sum of iron scores in the liver and greater intensity of staining in the spleen of ferric citrate versus control, and there were no differences in hemosiderin deposition in any other organs. The authors also noted no significant difference in serum

levels of aspartate aminotransferase or alanine aminotransferase in any of the treatment groups when compared to control and concluded that liver function was not affected by ferric citrate. They also concluded that the levels of iron accumulation in organs did not exceed acute toxic levels of iron reported in infants (20 mg/kg bw) or children and adults (200–300 mg/kg bw and 1400 mg/kg bw, respectively).

Fritz et al. (1975) summarized findings on the bioavailability of iron in iron-depleted male, weanling albino rats at eight different laboratories. Rats were depleted of iron for at least 21 days while on a low-iron basal diet, and blood was taken for hemoglobin analysis. Once depleted of iron (≤6 g hemoglobin/100 mL), animals were divided into 13 groups (n=≥8) and fed one of four diets (ferrous sulfate, ferric orthophosphate, 325-mesh hydrogen reduced iron, or ferric citrate at 16.0% iron). Rats were fed 6, 12, or 24 mg Fe/kg diet when on the ferric citrate diet. After two weeks of consuming the supplemented diets, blood was taken and again analyzed for hemoglobin. The average change in hemoglobin ranged from 0.26 to 5.39 g hemoglobin/100 mL with relative biological values (=100 x mg Fe from $FeSO_4$/mg Fe from dose) ranging from 74.7 to 125.3 and an average of 96 for the ferric citrate–treated animals. The iron supplementation induced an increase in hemoglobin, which is an essential protein in red blood cells that delivers and maintains adequate levels of oxygen in tissues, especially when anemic (Billett, 1990; Abbaspour et al., 2014).

Short-Term Oral Toxicity

Iida et al. (2020) fed six-week-old male Sprague Dawley rats 0.3% or 3% ferric citrate (65.1 mg iron or 526.8 mg iron per 100 g diet, respectively) for 11 days. Blood and urine samples were collected to determine iron status and phosphorus metabolism. There were no changes in food consumption for either group compared to control, and no anemia-related or phosphorus metabolism changes were observed in the 0.3% group when compared to control. Changes observed in the 3% group versus control were: significantly higher levels of serum iron, serum calcium, transferrin saturation (TSAT), and urinary excretion of calcium; and significantly lower unsaturated iron binding capacity (UBIC), serum phosphorus level, excretion amount of phosphorus, and iGF23 blood concentration. No changes were observed in the 3% ferric citrate–treated group for hemoglobin, red blood cell (RBC), total iron binding capacity (TIBC), serum creatinine, and urinary excretion of creatinine. The authors concluded that 3% ferric citrate could affect iron status, because it has inhibitory effects on phosphorus absorption and can have hematopoietic effects, which was not seen in the 0.3% ferric citrate–treated group. No other adverse effects or observations were noted by the authors of this study.

Pallarés et al. (1996) fed male Wistar albino rats (n=12/group) a ferric citrate diet (containing 45 mg iron/kg diet; equivalent to approximately 4.5 mg iron/kg bw/day) for 40 days. Although the main objective of the study was to determine iron replenishment from dietary iron supplementation in iron-deficient rats, the control group consisted of normal rats (non-iron-deficient) fed the same iron-supplemented diets. Food intake; change in body weight; serum iron concentration; intake and fecal excretion of iron, calcium, phosphate, and magnesium; and concentrations of these elements in the liver, femur, and sternum were measured. The authors did note that ferric citrate is more poorly absorbed than other forms of iron (e.g., ascorbate or sulfate); however, no adverse effects or

observations were noted for any of the parameters measured by the authors in the control ferric citrate group.

Based on an abstract only, Yokoi et al. (2018) conducted a study to evaluate the dose-effect relationship between dietary iron and hematological parameters in three-week-old male Wistar rats (n=54). Rats were divided into nine groups and were fed for five weeks on graded levels of supplemental dietary ferric citrate at 0, 7, 14, 21, 28, 35, 49, 126, and 252 mg iron/kg diet (approximately 0, 0.7, 1.4, 2.1, 2.8, 4.9, 12.6, 25.2 mg/kg bw/day, respectively). Blood samples were taken and analyzed for hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red blood cell (RBC) counts, and reticulocyte counts. There were no significant differences in parameters between the two highest doses, 126 and 252 mg/kg; however, the following significant differences were noted: increased hemoglobin, hematocrit, MCV, and MCH in the 126 mg/kg group when compared to doses below 49 mg/kg; increased RBC in the 126 mg/kg group when compared to doses below 7 mg/kg; and decreased reticulocytes in the 126 mg/kg group when compared to doses below 14 mg/kg. The authors concluded that the dietary iron level required to affect hemoglobin and red cell counts is between 49 mg/kg and 126 mg/kg in rats. No other adverse effects or observations were noted by the authors of the study.

Lau et al. (2018) fed normal and chronic kidney disease (CKD) Sprague-Dawley rats (8 weeks old) either a regular or 4% ferric citrate–supplemented diet (approximately 4000 mg/kg bw/day) for 6 weeks to study the effects of the gut microbiome on CKD. Blood, urine, fecal, and cecal samples were collected. No significant difference in systolic blood pressure, body weight, plasma phosphorus, plasma calcium, hemoglobin, blood urea nitrogen, plasma creatinine, and creatinine clearance were noted by the authors in the normal control animals on the regular diet versus normal control animals on the diet supplemented with ferric citrate. As expected, an increase in plasma total iron in rats supplemented with ferric citrate (285.0±19.1 μg/dl) compared to rats on normal diet (218.0±19.1 μg/dl) was observed.

Subchronic Oral Toxicity

Inai et al. (1994) conducted a 13-week study administering 8-week-old male and female $B6C3F_1$ mice (n = 75 males/72 females) dose of 0.06%, 0.12%, 0.25%, 0.5%, and 1% ferric citrate in their drinking water. In the 1% ferric citrate group, five males and seven females died by week four, and histology demonstrated that doses higher than 0.12% induced atrophy of liver cells or atrophy of lymphoid tissue in the spleen or thymus. The authors indicated that the findings were consistent with atrophy seen during starvation and not likely from ferric citrate treatment. No other mice died in any other treatment groups, and no significant increase in serum ferritin was observed in any treated groups. The only change noted was the average rates of body weight gain in the 0.25%, 0.5%, and 1% treated groups were less than 10% when compared to control, and thus, the maximum tolerated dose was determined to be 0.12%, which was the dose used in a subsequent chronic study.

Toyoda et al. (2014) conducted a 13-week oral subchronic study in F344 rats. Rats (n=40/sex) were randomly divided into four groups and given either a control or one of three doses of ferric citrate (0.25%, 1.0%, or 4.0%) in their diet. All animals survived the 13 weeks to necropsy, and no clinical signs were noted in any of the animals. There was severe enlargement of the right kidney in one of the female rats in the control group, which was characterized as nephroblastoma; it was deemed an incidental case, because it was in the untreated group, and similar lesions were not found in any other animals. Therefore, the animal was not included in the analyses. The following significant changes were observed: reduction of body weight gain in both sexes of the 4.0% group; decrease of red blood cells (RBC) and lymphocytes in both sexes of the 4.0% group; increase of platelets and eosinophils in both sexes of the 4.0% group; increase of mean corpuscular volume (MCV) in the 1.4% and 4.0% groups; increase of mean corpuscular hemoglobin (MCH) in the 4.0% group; increase in serum iron levels and decrease of total protein (TP) and transferrin in both sexes of the 4.0% group; decrease of transferrin in 1.0% females; increase of sodium (Na) and decrease of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in 4.0% males; increase of inorganic phosphorus (I)P and decrease of albumin (Alb) and chlorine (Cl) in the 4.0% females. There was a significant decrease in absolute heart weights and increase in the relative brain, spleen, adrenals, kidney, and testes weights in the 4.0% male group when compared to control. Significant decrease in the absolute and relative heart weights and increase in absolute and relative spleen weights were observed in the 4.0% females. An increase of absolute and relative liver weights was also observed in the 0.25% and 4.0% female group, respectively. Histopathology indicated a significant increase in hemosiderosis in the spleen; inflammation with eosinophilic infiltration and mucosal hyperplasia in the colon; infiltration of eosinophils, plasmacytosis, and hemosiderosis in the mesenteric lymph node; and increased hematopoiesis in the bone marrow in both sexes at the 4% treatment. There were several lesions in other organs detected sporadically; however, they were not significant nor attributed to treatment. Based on these reported findings, the authors estimated the NOAEL to be 1.0% in the diet (595.9 mg/kg-bw/day for males and 601.4 mg/kg-bw/day for females, respectively).

Luo et al. (2020) studied the intestinal effects in C57BL/6 mice (n =80) given ferric citrate at three different oral doses (2.5 mg/day (1.25%; 83.3 mg/kg bw/day), 5 mg/day (2.5%; 166.6 mg/kg bw/day), and 10 mg/day (5.0%; 333.3 mg/kg bw/day) for 16 weeks. Body weights and food consumption were measured every other week, and no significant difference in either was seen among the groups. Blood, jejunum, liver, spleen, heart, and lung samples were collected for further analysis. Iron overload was seen in all treatment groups, because iron levels in the blood serum, liver, spleen, heart, kidney, and jejunum were significantly increased in a dose-dependent manner when compared to control, with iron accumulation highest in the jejunum. Histology of the jejunum did not show any obvious pathological lesions in any of the groups; however, there were significant decreases in the 2.5% and 5% treated groups in villus height, villus height and crypt depth ratio, number of intraepithelial lymphocytes, and number of goblet cells when compared to control. Up-regulation of pro-inflammatory cytokines (IL-1β, IL-2, IL-6, and TNF-α) and malondialdehyde (MDA); down-regulation of anti-inflammatory cytokines (IL-4 and IL-10) and total antioxidation capacity (T-AOC), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH); increase in serum

D-lactate; and decrease in tight junction proteins (claudin-1, occluding, ZO-1), MUC-2, and TFF3 were noted. The authors concluded that long-term intake of ferric citrate at an oral dose at or above 166.6 mg/kg (249.9 mg/kg bw/day) in mice can cause iron overload, inducing intestinal oxidative stress in the jejunum and leading to impairment of the mucosal barrier and immune system function.

Chronic Toxicity/Carcinogenicity

Inai et al. (1994) conducted a 96-week study in 8-week-old male and female $B6C3F_1$ mice (n = 150/sex) and divided into three groups, each consisting of 50 males and 50 females and administered distilled drinking water (control) or water with 0.06% or 0.12% ferric citrate *ad libitum*. Doses were based on a subacute toxicity test in which 0.12% was determined to be the maximum tolerated dose. After the 96-week treatment period, all surviving animals were given a four-week recovery period of distilled water and basal diet. All animals that died during the study and animals that survived the entire 100-week period were subject to examination and autopsy. Tumors were examined microscopically and weighed. There were no significant changes in average water intake (5.6–6.6 mL/day for males and 3.8–4.3 mL/day for females, respectively) and average body weights (28.1±9.0 – 37.1±6.3 g for males and 28.1±9.0 – 37.1±6.3 g for females, respectively) compared to controls. The average time of survival in the treated male mice was shorter than in the control group. No differences in survival were seen in the female mice. The first mouse that died with a tumor occurred at week 59. The incidence of tumors, specifically in the liver, in the 0.12% treated male group was significantly less than the control group, and there were no significant differences in incidence or distribution of tumors in female mice. Tumors in male mice were predominantly hepatocellular, with an inverse dose-response; however, when using a "time-adjusted analysis," no significant dose-relationship could be determined. The predominant tumor in female mice was lymphoma/lymphoid leukemia, but there was no significant difference in incidence between the groups. Other tumors (malignant lymphoma/lymphoid leukemia, bronchiolo-alveolar adenoma, and carcinoma of the lung) observed in male mice were not associated with ferric citrate treatment, and a low incidence of all other tumors was noted in female mice. Amyloidosis affecting the spleen, liver, and kidney was observed in males, but was not significant in incidence between the three groups and was not attributed to ferric citrate treatment. Hemosiderosis was seen in all groups, but no fibrosis of the liver or pancreas was observed. No other pathological changes caused by iron deposition were observed in any of the treatment groups. Taken together, the authors concluded that the oral administration of ferric citrate at 0.12% (180 mg/kg bw/day) in drinking water had no significant effect on tumor incidence or distribution in mice.

Wyllie et al. (1998) studied the role of iron in estrogen-induced carcinogenesis in male Syrian hamsters when fed a low-iron (3.9 ppm Fe as ferric citrate; approximately 4.68 mg Fe/kg bw/day) or high-iron (384 ppm Fe as ferric citrate; approximately 46 mg Fe/kg bw/day) diet for five months. There was no significant difference in average body weight in low- or high-iron diets when compared to normal rodent chow (215 ppm Fe). Neither a low- nor a high-iron diet induced renal tumors or affected total serum iron concentration, total iron binding capacity, or kidney and liver non-heme iron. Histological examination of

the liver revealed no iron deposits in the low-iron-diet group, but some iron deposits in hepatocytes of the high-iron-diet group were noted. In the kidney, there was no stainable iron in the low- or high-iron-diet groups. No other adverse effects were noted.

Mutagenicity/Genotoxicity

Ishidate et al. (1984) tested more than 200 food additives for mutagenicity using the Ames assay and chromosomal aberration *in vitro* assay. Among these additives, ferric citrate was tested; however, the CAS number and purity were unknown. Ferric citrate was negative in the Ames assay, which was conducted in *S. typhimurium* strains TA92, TA1535, TA100, TA1537, TA94, and TA98 at a maximum dose of 25 mg/plate with and without S-9 activation. The chromosomal aberration *in vitro* assay in a Chinese hamster fibroblast cell line was also negative at the maximum dose of 0.5 mg/mL. The authors concluded that no evidence of genotoxicity or mutagenicity was observed in either assay, up to the highest dose of ferric citrate tested.

Hartwig et al. (1995) evaluated the potential of ferric citrate to induce DNA damage in V79 Chinese hamster cells. Neither DNA strand breaks nor formamidopyrimidine-DNA glycosylase (Fpg) were detected when treated with up to 2 mM of ferric citrate for 24 or 48 hours.

Human Studies

Rao et al. (1978) evaluated iron absorption in healthy male (n=24) and female (n=11) volunteers when given radiolabeled ferrous citrate mixed uniformly with coarse-ground crude cooking salt at 1000 ppm Fe, either alone or in a rice-based meal. Iron absorption was 23.1% in men and 31.1% in women when given alone and 5.2% in men and 12.3% in women when given in a meal. The authors noted that ferrous citrate can be used as a good source of iron fortification in salt, because it has good bioavailability when compared to other forms of iron (e.g., ferrous sulfate). No adverse events related to ferrous citrate consumption were reported.

Safety Summary

The above data support the conclusion that the consumption of iron citrate used as an anti-caking agent in salt is not expected to have any toxicological concerns. The existing information described above addresses all toxicological endpoints that are relevant to the human oral consumption of iron citrate (e.g., ADME, short-term and subchronic oral toxicity, chronic toxicity/carcinogenicity, and genotoxicity/mutagenicity). In addition, the constituents of iron citrate are consumed as part of a normal human diet and are naturally present or routinely added to foods. Potential intake of citrate is well below what would be expected from normal dietary levels from natural sources such as fruit juices. Upper intake limits (ULs) of 40 mg/day for children (1–13 years old) and 45 mg/day adults (>14 years old) have been established for iron by the Food and Nutrition Board of the National Institute of Medicine, as well as a provisional maximum tolerable daily intake (PMTDI) for iron established by JECFA of 0.8 mg/kg-bw/day, equivalent to 56 mg/day based on a 70-kg individual. The potential iron intake resulting from iron citrate as an anti-caking

agent in salt (0.22-0.26 mg/person/day) is well below the ULs and PMTDI. Therefore, it can be concluded that the publicly available data/information on iron citrate and related compounds that have been reviewed as part of this current GRAS assessment are sufficient to support the safe use of iron citrate for the proposed anti-caking use in salt.

Basis for the GRAS Determination

Introduction

The regulatory framework for determining whether a substance can be considered GRAS in accordance with section 201(s) (21 U.S.C. § 321(s)) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. § 301 et. Seq.) ("the Act"), is set forth at 21 CFR 170.30, which states:

> General recognition of safety may be based only on the view of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. The basis of such views may be either (1) scientific procedures or (2) in the case of a substance used in food prior to January 1, 1958, through experience based on common use in food. General recognition of safety requires common knowledge about the substance throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food.
>
> General recognition of safety based upon scientific procedures shall require the same quantity and quality of scientific evidence as is required to obtain approval of a food additive regulation for the ingredient. General recognition of safety through scientific procedures shall ordinarily be based upon published studies, which may be corroborated by unpublished studies and other data and information.

These criteria are applied in the analysis below to determine whether the use of the iron citrate ingredient in salt that is the subject of this GRAS determination is GRAS based on scientific procedures. All data relied upon in this GRAS determination are publicly available and generally known, and therefore meet the "general recognition" standard under the Federal Food, Drug, and Cosmetics Act.

Safety Determination

The Cargill iron citrate ingredient that is the subject of the current GRAS determination is proposed for use as an anti-caking agent in salt at a use level ≤135 ppm (or 30 ppm calculated as iron). The iron citrate product will be used as an alternative to, not in addition to, other anti-caking agents used in salt.

Numerous toxicology studies employing the proposed iron citrate ingredient have been conducted and published and provide support for the safety of the intended use of the ingredient. These studies include *in vitro* studies and *in vivo* animal studies (i.e., short-term

and subchronic toxicity, mutagenicity and genotoxicity, and chronic toxicity/ carcinogenicity).

General Recognition of the Safety of Iron Citrate

The intended use of iron citrate in salt has been determined to be safe through scientific procedures, as set forth in 21 CFR § 170.3(b), thus satisfying the so-called "technical" element of the GRAS determination, and this determination is based on the following:

- The Cargill iron citrate product that is the subject of this GRAS determination is a complexation product of iron and sodium citrate. It is a dark green/red aqueous solution intended for use as an anti-caking agent in salt.
- Iron citrate is prepared from a ferrous iron salt (1.0 molar equivalent Fe) and sodium citrate (1.0 molar equivalent), with sodium hydroxide (0.5–1.2 molar equivalents) added for pH adjustment. The iron source can be any food-grade ferrous iron salt such as iron (II) sulfate, iron (II) chloride, or a mixture of these iron salts.
- The iron citrate is intended for use as an anti-caking agent in salt at a use level ≤135 ppm (or 30 ppm calculated as iron).
- In 2001, IOM established recommended daily allowances (RDAs) and tolerable upper intake limits (ULs) for different populations in the United States. The RDA for iron is 8 mg/day for all groups of men and postmenopausal women and 18 mg/day for premenopausal women. ULs of 40 mg/day for children (1–13 years old) and 45 mg/day adults (>14 years old) was established.
- Based on the maximum use level of 135 ppm of the proposed anti-caking agent (equivalent to 30 ppm iron), the potential daily exposure to iron citrate and iron ranges from 0.98 to 1.18 mg//person/day and 0.22 to 0.26 mg/person/day, respectively.
- The calculated iron exposure of an individual from use of the iron citrate product as an anti-caking agent in salt (range of 0.22–0.26 mg/person/day) is low, and the contribution to background dietary intakes of iron would be negligible because the mean iron intake in the United States is approximately 16 to 18 mg/day for men and 12 mg/day for women; 90th percentile intake was approximately 25 to 31 mg/day for men and 18 to 20 mg/day for women (IOM, 2001).
- Numerous toxicology studies employing iron citrate have been conducted and published and provide support for the safety of the intended use of the iron citrate ingredient. These studies include *in vitro* studies and *in vivo* animal studies (i.e., short-term and subchronic toxicity, mutagenicity and genotoxicity, and chronic toxicity/carcinogenicity).

- Potential intake of citrate is well below what would be expected from normal dietary levels from natural sources such as fruit juices.
- The body of publicly available scientific literature on the consumption and safety of iron citrate is sufficient to support the safety and GRAS determination of the proposed use of the iron citrate ingredient.

Because this safety evaluation was based on generally available and widely accepted data and information, it also satisfies the so-called "common knowledge" element of a GRAS determination.

Determination of the safety and GRAS status for the intended use of the iron citrate ingredient that is the subject of this evaluation has been made through the deliberations of an Expert Panel convened by Cargill and composed of Michael Carakostas, DVM, Ph.D.; Stanley M. Tarka, Jr., Ph.D., F.A.T.S.; and Thomas A. Vollmuth, Ph.D. These individuals are qualified by scientific training and experience to evaluate the safety of substances intended to be added to foods. They have critically reviewed and evaluated the publicly available information summarized in this document and have individually and collectively concluded that iron citrate, produced in a manner consistent with GMP and meeting the specifications described herein, is safe under its intended conditions of use. The Panel further concludes that the use of iron citrate is GRAS based on scientific procedures, and that other experts qualified to assess the safety of foods and food additives would concur with these conclusions. The Panel's GRAS opinion is included as Exhibit 1 to this document.

It is also Cargill's opinion that other qualified scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Cargill has concluded that the iron citrate ingredient is GRAS under the intended conditions of use on the basis of scientific procedures; and 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.

Cargill is not aware of any information that would be inconsistent with a finding that the proposed use of the iron citrate ingredient in food for human consumption, meeting appropriate specifications, and used according to cGMP, is GRAS. Recent reviews of the scientific literature revealed no potential adverse health concerns.

§ 170.250 Part 7, Supporting Data and Information

The following references are all generally available, unless otherwise noted. Appendix A (analytical Certificates of Analysis and additional analytical data) and Exhibit 1 (the signed Expert Panel report) are not generally available but are attached for reference.

References

Abbaspour N, Hurrell R, Kelishadi R. 2014. Review on iron and its importance for human health. J Res Med Sci 19(2):164–174.

Abrahamson HB, Rezvani AB, Brushmiller JG. 1994. Photochemical and spectroscopic studies of complexes, of iron(III) with citric acid and other carboxylic acids. Inorganica Chimica Acta 226:117-127.

Billett HH. 1990. Hemoglobin and hematocrit. In: Walker HK, Hall WD, Hurst JW (eds). Clinical Methods: The History, Physical, and Laboratory Examinations. 3rd edition. Chapter 151. Butterworth Publishers, a division of Reed Publishing.

Clark AC, Prenzler PD, Scollarly GR. 2007. Impact of the condition of storage of tartaric acid solutions on the production and stability of glyoxylic acid. Food Chemistry 102(3):905-916.

Ems T, St Lucia K, Huecker MR. 2021. Biochemistry, Iron Absorption. [Updated 2021 Apr 26]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. Available from: https://www.ncbi.nlm.nih.gov/books/NBK448204/.

FDA (U.S. Food and Drug Administration). 2004. GRN 152. GRAS Notification for sodium iron EDTA. Prepared by Kraft.

FDA (U.S. Food and Drug Administration). 2006. GRN 178. GRAS Notification for sodium iron EDTA. Prepared by AKZO Nobel Chemicals.

FDA (U.S. Food and Drug Administration). 2009. GRN 271. GRAS Notification for ferrous ammonium phosphate. Prepared by Nestle USA, Inc.

FDA (U.S. Food and Drug Administration). 2013. GRN 441. GRAS Notification for sodium ferrous citrate. Prepared by Eisai Food & Chemical Co., Ltd.

FDA (U.S. Food and Drug Administration). 2015. GRN 532. GRAS Notification for complexation products of sodium tartrates with iron (III) chloride. Prepared by AKZO Nobel Industrial Chemicals BV.

FDA (U.S. Food and Drug Administration). 2021. GRN 959. GRAS Notification for iron milk proteinate. Prepared by Societe des Produits Nestle S.A.

Fiume MZ, & Cosmetic Ingredients Review (CIR) Expert Panel. 2003. Final report on the safety assessment of triacetin. Int J Toxicol 22 Suppl 2:1–10.

Floege J, Funk F, Ketteler M, Rastogi A, Walpen S, Covic AC, Sprague SM. 2020. Iron kinetics following treatment with sucroferric oxyhydroxide or ferric citrate in healthy rats and models of anaemia, iron overload or inflammation. Nephrology, dialysis, transplantation: Official publication of the European Dialysis and Transplant Association — European Renal Association 35(6):946–954.

Fritz JC, Pla GW, Harrison BN, Clark GA. 1975. Estimation of the bioavailability of iron. J Assoc Official Analyt Chemists 58(5):902–905.

Hartwig A, Schlepegrell R. 1995. Induction of oxidative DNA damage by ferric iron in mammalian cells. Carcinogenesis 162(12):3009–3013.

Iida A, Matsushita M, Ohta T, Yamada T. 2020. Conventional and novel impacts of ferric citrate on iron deficiency anemia and phosphorus metabolism in rats. J Vet Med Sci 82(3):379–386.

Inai K, Fujihara M, Yonehara S, Kobuke T. 1994. Tumorigenicity study of ferric citrate administered orally to mice. Food Chem Toxicol 32(6):493–498.

IOM (Institute of Medicine). 2001. Iron. In: Dietary Reference Intakes: For Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. National Academy of Sciences, Panel on Micronutrients, Food and Nutrition Board, IOM. Washington, DC: National Academy Press (NAP), pp. 290-393. Available at: http://www.nap.edu/openbook.php?isbn=0309072794.

Ishidate M, Jr, Sofuni T, Yoshikawa K, Hayashi M, Nohmi T, Sawada M, Matsuoka A. 1984. Primary mutagenicity screening of food additives currently used in Japan. Food Chem Toxicol 22(8):623–636.

JECFA. 1974. Citric acid and its Calcium, Potassium, and Sodium Salts. In: Toxicological Evaluation of Some Food Additives Including Anticaking Agents, Antimicrobials, Antioxidants, Emulsifiers and Thickening Agents. 17th Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), June 25-July 4, 1973, Geneva, Switz. (WHO Food Additives Series no 5). Food and Agriculture Organisation of the United Nations (FAO). Geneva, Switz.: World Health Organization (WHO). Available at: https://inchem.org/documents/jecfa/jecmono/v05je24.htm.

JECFA. 1983. Iron. In: Toxicological Evaluation of Certain Food Additives and Contaminants. 27th Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), Apr. 11-20, 1983, Geneva, Switz. (WHO Food Additives Series, no 18). Geneva, Switz.: World Health Organization (WHO), pp. 203-219. Available at: http://www.inchem.org/documents/jecfa/jecmono/v18je18.htm.

Kuether CA, Smith AH. 1941. The absorption and fate of free citric acid in the rat. J Biol Chem 137(2):647–658.

Lau WL, Vaziri ND, Nunes ACF, Comeau AM, Langille MGI, England W, Khazaeli M, Suematsu Y, Phan J, Whiteson K. 2018. The phosphate binder ferric citrate alters the gut microbiome in rats with chronic kidney disease. J Pharmacol Exper Therapeut 367(3):452–460.

Luo Q, Lao C, Huang C, Xia Y, Ma W, Liu W, Chen Z. 2021. Iron overload resulting from the chronic oral administration of ferric citrate impairs intestinal immune and barrier in mice. Biol Trace Element Res 199(3):1027–1036.

Pallarés I, López-Aliaga I, Lisbona F, Moratalla A, Gómez-Ayala AE, Barrionuevo M, Hartiti S, Alférez MJ, Campos MS. 1996. Effects of iron replenishment on iron, calcium, phosphorus and magnesium metabolism in iron-deficient rats. Int J Vitamin Nutr Res 66(2):158–165.

Rao BS, Kathoke S, Apte SV. 1978. Mono ferrous acid citrate (FeC6O7H2O) as an iron fortificant. Brit J Nutr 39(3):663–665.

SCOGS. 1980. Select Committee on GRAS Substances (SCOGS) Opinion: Iron and Iron Salts. SCOGS Report No. 35. NTIS Accession Number PB80178676.

Toyoda T, Cho Y, Mizuta Y, Akagi J, Ogawa K. 2014. A 13-week subchronic toxicity study of ferric citrate in F344 rats. Food Chem Toxicol 74:68–75.

U.S. FDA. 1959. Appraisal of the safety of chemicals in foods, drugs and cosmetics, pp. 1-107. Washington, DC: Association of Food and Drug Officials of the United States.

U.S. FDA. 2012. U.S. Code of Federal Regulations (CFR). Title 21—Food and Drugs (Food and Drug Administration). Washington, DC: U.S. Government Printing Office (GPO). Available at: http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR.

Wallace TC, Cowan AE, Bailey RL. 2019. Current sodium intakes in the United States and the modeled effects of glutamate incorporation into select savory products. Nutrients 11:2691.

Wyllie S, Liehr JG. 1998. Enhancement of estrogen-induced renal tumorigenesis in hamsters by dietary iron. Carcinogenesis 19(7):1285–1290.

Yokoi K, Konomi A. 2018. Dose-effect relationship between dietary iron and hematological parameters in rats. FASEB Journal. Available at: https://doi.org/10.1096/fasebj.31.1_supplement.801.5

APPENDIX A

Certificates of Analysis and Analytical Results

APPENDIX B

Stability Testing Report

EXHIBIT 1

Report of the Expert Panel

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

GENERALLY RECOGNIZED AS SAFE *(GRAS)* NOTICE (Subpart E of Part 170)

Form Approved: OMB No. 0910-0342; Expiration Date: 07/31/2022
(See last page for OMB Statement)

FDA USE ONLY

GRN NUMBER	DATE OF RECEIPT
001036	Oct 26, 2021
ESTIMATED DAILY INTAKE	INTENDED USE FOR INTERNET
NAME FOR INTERNET	
KEYWORDS	

Transmit completed form and attachments electronically via the Electronic Submission Gateway *(see Instructions)*; OR Transmit completed form and attachments in paper format or on physical media to: Office of Food Additive Safety *(HFS-200)*, Center for Food Safety and Applied Nutrition, Food and Drug Administration,5001 Campus Drive, College Park, MD 20740-3835.

SECTION A – INTRODUCTORY INFORMATION ABOUT THE SUBMISSION

1. Type of Submission *(Check one)*

☒ New ☐ Amendment to GRN No. _____ ☐ Supplement to GRN No. _____

2. ☒ All electronic files included in this submission have been checked and found to be virus free. *(Check box to verify)*

3 Most recent presubmission meeting *(if any)* with FDA on the subject substance *(yyyy/mm/dd):* _____

4 For Amendments or Supplements: Is your amendment or supplement submitted in response to a communication from FDA? *(Check one)* ☐ Yes ☐ No If yes, enter the date of communication *(yyyy/mm/dd):* _____

SECTION B – INFORMATION ABOUT THE NOTIFIER

1a. Notifier

Name of Contact Person: Witty Brathwaite		Position or Title: Principal Scientist, Scientific and Regulatory Affairs	
Organization *(if applicable)*: Cargill, Inc.			
Mailing Address *(number and street)*: 15407 McGinty Road West			
City: Wayzata	State or Province: Minnesota	Zip Code/Postal Code: 55391	Country: United States of America
Telephone Number: 647-464-8081	Fax Number:	E-Mail Address: Witty_Brathwaite@cargill.com	

1b. Agent or Attorney *(if applicable)*

Name of Contact Person: Donald Schmitt		Position or Title: Senior Managing Scientist	
Organization *(if applicable)*: ToxStrategies			
Mailing Address *(number and street)*: 739 Thornapple Drive			
City: Naperville	State or Province: Illinois	Zip Code/Postal Code: 60540	Country: United States of America
Telephone Number: 630-352-0303	Fax Number:	E-Mail Address: dschmitt@toxstrategies.com	

SECTION C – GENERAL ADMINISTRATIVE INFORMATION

1. Name of notified substance, using an appropriately descriptive term

Complexation products of iron with sodium citrate

2. Submission Format: *(Check appropriate box(es))*

☒ Electronic Submission Gateway

☐ Electronic files on physical media

☐ Paper

If applicable give number and type of physical media

3. For paper submissions only:

Number of volumes _____

Total number of pages _____

4. Does this submission incorporate any information in CFSAN's files? *(Check one)*

☒ Yes *(Proceed to Item 5)* ☐ No *(Proceed to Item 6)*

5. The submission incorporates information from a previous submission to FDA as indicated below *(Check all that apply)*

☒ a) GRAS Notice No. GRN 000152

☐ b) GRAS Affirmation Petition No. GRP _____

☐ c) Food Additive Petition No. FAP _____

☐ d) Food Master File No. FMF _____

☒ e) Other or Additional *(describe or enter information as above)* GRNs 000178, 000271, 000441, 000532, 000959

6. Statutory basis for conclusions of GRAS status *(Check one)*

☒ Scientific procedures *(21 CFR 170.30(a) and (b))* ☐ Experience based on common use in food *(21 CFR 170.30(a) and (c))*

7. Does the submission (including information that you are incorporating) contain information that you view as trade secret or as confidential commercial or financial information? (see 21 CFR 170.225(c)(8))

☐ Yes *(Proceed to Item 8*

☒ No *(Proceed to Section D)*

8. Have you designated information in your submission that you view as trade secret or as confidential commercial or financial information *(Check all that apply)*

☐ Yes, information is designated at the place where it occurs in the submission

☐ No

9. Have you attached a redacted copy of some or all of the submission? *(Check one)*

☐ Yes, a redacted copy of the complete submission

☐ Yes, a redacted copy of part(s) of the submission

☐ No

SECTION D – INTENDED USE

1. Describe the intended conditions of use of the notified substance, including the foods in which the substance will be used, the levels of use in such foods, and the purposes for which the substance will be used, including, when appropriate, a description of a subpopulation expected to consume the notified substance.

Intended for use as an anti-caking agent in salt at a use level of up to 135 ppm (or 30 ppm calculated as iron).

2. Does the intended use of the notified substance include any use in product(s) subject to regulation by the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture?

(Check one)

☐ Yes ☒ No

3. If your submission contains trade secrets, do you authorize FDA to provide this information to the Food Safety and Inspection Service of the U.S. Department of Agriculture?

(Check one)

☐ Yes ☐ No , you ask us to exclude trade secrets from the information FDA will send to FSIS.

SECTION E – PARTS 2 -7 OF YOUR GRAS NOTICE

(check list to help ensure your submission is complete – PART 1 is addressed in other sections of this form)

☒ PART 2 of a GRAS notice: Identity, method of manufacture, specifications, and physical or technical effect (170.230).

☒ PART 3 of a GRAS notice: Dietary exposure (170.235).

☒ PART 4 of a GRAS notice: Self-limiting levels of use (170.240).

☒ PART 5 of a GRAS notice: Experience based on common use in foods before 1958 (170.245).

☒ PART 6 of a GRAS notice: Narrative (170.250).

☒ PART 7 of a GRAS notice: List of supporting data and information in your GRAS notice (170.255)

Other Information

Did you include any other information that you want FDA to consider in evaluating your GRAS notice?

☐ Yes ☒ No

Did you include this other information in the list of attachments?

☐ Yes ☐ No

SECTION F – SIGNATURE AND CERTIFICATION STATEMENTS

1. The undersigned is informing FDA that Cargill

(name of notifier)

has concluded that the intended use*(s)* of complexation products of iron with sodium citrate

(name of notified substance)

described on this form, as discussed in the attached notice, is (are) not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on your conclusion that the substance is generally recognized as safe recognized as safe under the conditions of its intended use in accordance with § 170.30.

2. Cargill *(name of notifier)* agrees to make the data and information that are the basis for the conclusion of GRAS status available to FDA if FDA asks to see them; agrees to allow FDA to review and copy these data and information during customary business hours at the following location if FDA asks to do so; agrees to send these data and information to FDA if FDA asks to do so.

739 Thornapple Drive, Naperville, IL 60540

(address of notifier or other location)

The notifying party certifies that this GRAS notice is a complete, representative, and balanced submission that includes unfavorable, as well as favorable information, pertinent to the evaluation of the safety and GRAS status of the use of the substance.The notifying party certifies that the information provided herein is accurate and complete to the best or his/her knowledge. Any knowing and willful misinterpretation is subject to criminal penalty pursuant to 18 U.S.C. 1001.

3. Signature of Responsible Official, Agent, or Attorney	Printed Name and Title	Date (mm/dd/yyyy)
	Donald F. Schmitt, Senior Managing Scientist	10/26/2021

SECTION G – LIST OF ATTACHMENTS

List your attached files or documents containing your submission, forms, amendments or supplements, and other pertinent information. Clearly identify the attachment with appropriate descriptive file names (or titles for paper documents), preferably as suggested in the guidance associated with this form. Number your attachments consecutively. When submitting paper documents, enter the inclusive page numbers of each portion of the document below.

Attachment Number	Attachment Name	Folder Location (select from menu) (Page Number(s) for paper Copy Only)
	Form3667.pdf	Administrative
	toFDAFINALCargillironcitrateGRASNotice.pdf	Administrative
	toFDAFINALCargillironcitrateGRASNotice.pdf	GRAS Notice
	AppendixACOAs.pdf	GRAS Notice
	AppendixBStabilityReport.pdf	GRAS Notice
	Exhibit1102521.pdf	GRAS Notice

CHEMISTRY

1. Cargill provides a general description on p. 6 of the complexation products of iron with sodium citrate and states the chemical formula to be $FeC_6H_5O_6Na$. We note that there are no existing Food Chemical Codex (*FCC)* monographs or Chemical Abstracts Service (CAS) Registry number for Cargill's complexation product. Please clarify the chemical nomenclature, formula, structure, composition, iron species, and molar ratio of iron to citrate in Cargill's complexation products of iron with sodium citrate.

Response:

Cargill thanks the reviewer for their questions, and for pointing out this typo in the GRAS Notice. The corrected (nominal) empirical chemical formula for the active ingredient is $Na_{x+1}FeC_6H_{5-x}O_7$, where x = 0.5 to 1.0, with a molecular weight of 278.93 – 289.92 g/mol. Hence, the maximum level of use of iron citrate in Part 3 should also be adjusted to 156 ppm iron citrate (30 ppm Fe) based on the corrected molecular weight.

The corresponding intakes of iron citrate and iron in Part 3 are therefore revised as follows:

Current Intakes of Sodium and Salt in the U.S. (Wallace et al., 2019) and Corresponding Intakes of Iron Citrate and Iron from Intended Use as Anti-Caking Agent in Salt*

	Sodium (mg/day)	**Salt (mg/day)**	**Iron Citrate (mg/day)**	**Iron (mg/day)**
>1 year	3,361	8,402	1.30	0.25
1-3 years	2,063	5,156	0.83	0.16
1-18 years	2,906	7,265	1.14	0.22
≥19 years	3,499	8,748	1.35	0.26

*Maximum intended use level of 156 ppm iron citrate in salt (30 ppm iron)

Cargill's iron citrate product could be more precisely described as "complexation products of iron(II) and iron(III) with citrate ionization ≥ 3.5 but ≤ 4. The functionally active anti-caking formulation is prepared and used as a homogenous aqueous solution, where no discrete solid compound is isolated, and therefore no singular discrete chemical structure/formula is assigned. As prepared, the corrected (nominal) empirical chemical formula for the active ingredient is $Na_{x+1}FeC_6H_{5-x}O_7$, where x = 0.5 to 1.0. It contains iron in the iron(II) oxidation state, but it is known that solutions will oxidize to iron(III) over time with no loss in anti-caking effectiveness. The ratio of iron to (ionized) citrate is 1:1. The solution structure of the complexes have not been further characterized.

2. We note that "sodium ferrous citrate" is listed with two CAS numbers (CAS No. 43160-25-4 for $C_{12}H_{10}O_{14}FeNa_4$ and CAS No. 50717-86-7 for $C_6H_5FeNaO_7$). We further note that a physical description of sodium ferrous citrate (CAS No. 43160-25-4) is provided in the FCC 12 (2021) monograph. Please discuss how your complexation product of iron with sodium citrate is different from sodium ferrous citrate described in FCC 12 and from the sodium ferrous citrate with CAS No. 50717-86-7. We further note that the iron citrate ingredients in FCC and with established CAS numbers contain 7 oxygens, whereas your

chemical formula indicates 6 oxygens. Please clarify the chemical formula for your ingredient.

Response:

Cargill thanks the reviewer for pointing out this typo in the GRAS Notice. The corrected (nominal) empirical chemical formula for the active ingredient is $Na_{x+1}FeC_6H_{5-x}O_7$, where x = 0.5 to 1.0, with a molecular weight of 278.93 – 289.92 g/mol . As discussed above, the assigned chemical formula is a nominal representation of the active ingredient in solution based on the molar ratios of the ingredients used to prepare the solution. A formal elemental analysis is not provided because the functional anti-caking agent prepared is an aqueous solution, not a discrete isolated solid material.

The sodium ferrous citrate referenced in the FCC 12 monograph with CAS number 43160-25-4 is a 1:2 complex of Fe(II) and citrate. Cargill's iron citrate is 1:1 complex of Fe(II) with citrate. Sodium ferrous citrate with CAS number 50717-86-7 is a 1:1 complex of Fe(II) and specifically citrate with an ionization of 3 (*i.e.*, $C_6H_5O_7^{-3}$). Cargill's iron citrate is a 1:1 complex of Fe(II) with citrate which has been ionized to ≥ 3.5 and ≤ 4.

3. Cargill states that iron citrate is dark green/red in color (p. 8). Please clarify that at the intended use levels, iron citrate is not intended to impart color to salt. In addition, please clarify if the form of iron (i.e., ferric or ferrous) is responsible for the different colors of the final product or if there is something else that dictates the resulting color of the product.

Response:

Cargill does not intend to use iron citrate as a color additive at the levels proposed for use in salt. It is assumed that the change in color is due to only to oxidation of the iron(II) complexes to iron(III) complexes, which is consistent with the solution color remaining stable when protected from exposure to atmospheric oxygen.

4. Cargill states that the intended use of iron citrate is as an anti-caking agent in salt (p. 6). Please clarify whether the intended use is in a tabletop salt, salt used in food preparations, or both.

Response:

Cargill proposes the use of iron citrate as an anti-caking agent in the manufacture of both tabletop salt and salt used in food preparations.

5. Cargill provides analytical methods to support the proposed specifications for iron citrate (Table 1, p. 10). Please confirm that all analytical methods used to test for the specification parameters are validated for the stated purpose.

Response:

Cargill confirms that all analytical methods used to test for the specification parameters are widely accepted methods and have been validated for the stated purpose.

6. Cargill proposes a broad range of pH values (5-10) for the final iron citrate product (Table 1, p. 10). Please provide a rationale for why such a wide range of pH values are observed and indicate if there is a technical reason for the broad range of pH values.

Response:

The pH range of 5 to 10 is specified because pH can vary broadly depending on conditions (*i.e.*, solution concentration, choice of $FeCl_2$ or $FeSO_4$, ratio of NaOH to Fe, and oxidation of the solution over time) while still being effective across this pH range. Experimental data shows that solutions with pH > 5 are effective as an anti-caking agent. Solutions with pH < 5 are not sufficiently effective, and their efficacy decreases as the pH is lowered further. It is assumed that at pH > 10 precipitation of iron oxide/hydroxides would present an operational barrier to application onto the salt.

7. Cargill notes that the starting materials are food grade, and the substance is manufactured according to good manufacturing practices. However, we note that the 3 mg/kg lead specification in Table 1 and the corresponding batch analyses of 2.5-2.7 mg/kg, Table 2 are high. For comparison, the lead limit in the FCC 12 monographs for sodium ferrous citrate is 1 mg/kg and 2 mg/kg for ferrous citrate. The specifications for the other heavy metals were also high (≤ 1 mg/kg, Table 1) when the results from the batch analyses were listed as not detected (ND) using an analytical method with a limit of detection of 0.5 mg/kg. We suggest Cargill lower the specifications for heavy metals to be reflective of the batch analyses and provide a rationale for the high observed lead levels in the final iron citrate product.

Response:

It is likely that purification steps used in the preparation of sodium ferrous citrate and ferrous citrate (i.e., crystallization/precipitation and rinsing of the resulting solid product) allow residual heavy metals from the starting materials to be removed in the mother liquor that is rinsed away. Cargill's iron citrate is an aqueous solution used without further purification, which is the functionally active composition that performs as an anti-caking agent in salt. Cargill uses raw materials that comply with appropriate FCC 12 monographs, wherein residual limits for lead are established at 2 mg/kg for each of ferrous sulfate, sodium citrate, and sodium hydroxide. Even at the current specification limit, potential exposure to lead from the intended use of iron citrate is very low (4 ng/day; below 3 µg/day for children – FDA 2018 new maximum daily intake level). This is 25 to 50 times lower than potential exposure to lead (100-200 ng/day; based on estimated intakes reported in GRN 441) from consumption of sodium ferrous citrate as nutrient supplement. However, Cargill will continue to look into sourcing raw materials that contain lower residual lead levels.

As for the other heavy metals, given the FCC 12 monograph limits for arsenic (≤3 mg/kg), mercury (≤1 mg/kg), and cadmium (none specified) in the raw materials used to produce iron citrate, Cargill will maintain a specification of ≤1 mg/kg for each of these heavy metals. As discussed above, the intended use of iron citrate as an anti-caking agent in salt (156 ppm) is not expected to contribute significantly to heavy metal exposure in the diet. However, Cargill will continue to look into sourcing raw materials that would limit heavy metal levels in the final iron citrate product.

8. Cargill provides analytical results for a total of four batches in Tables 2 and 3 to support the provided specifications. However, on p. 9 the batch analyses are described as "three non-consecutive lots". Please clarify this discrepancy.

Response:

The description on p. 9 is in error and should read "four non-consecutive lots."

9. Please provide additional information (e.g., method of detection) about the ion chromatography method, and gravimetric analyses used for the analysis of certain specification parameters (Table 1).

Response:

Ion Chromatography was conducted using a conductivity detector (IC-CD). Gravimetric analysis was conducted on an independently certified analytical balance, capable of measuring to four decimal places. For the density measurements specifically, the weight of a 1000 µL sample, discharged onto the balance from a 100 – 1000 µL micropipette, was measured in triplicate and averaged for each solution.

TOXICOLOGY

1. Absorption, distribution, metabolism, and excretion (ADME) information is provided for citrates or citric acid in the notice (p 19). However, the ADME information provided for the iron component of the ferrous citrate (iron (II) citrate) in non-iron-depleted subjects is incomplete.
    a. Please briefly describe all aspects of the ADME for the iron in the ferrous citrate ingredient and discuss that the proposed intake/exposure is not expected to increase body's iron burden in individuals with no iron deficiency.

Response:

Iron is present in the body, primarily as part of hemoglobin in erythrocytes. Four major classes or iron-containing proteins exist in humans: iron-containing heme proteins, iron-sulfur enzymes, proteins for iron storage and transport, and other iron-containing or activated enzymes. The body highly conserves iron content. Iron balance is maintained by the regulation of absorption in the upper small intestine. There are two pathways for the absorption of iron in humans; 1) the uptake of heme iron derived primarily from hemoglobin and myoglobin in meat and 2) the absorption of

non-heme iron, primarily as iron salts, that are extracted from plant and dairy foods and become soluble in the lumen of the stomach and duodenum. Most of the iron consumed by humans is in the latter non-heme form.

IOM (2001) states that non-heme iron (such as ferric and ferrous citrate) absorption depends on the solubilization of predominately ferric food iron in the acid milieu of the stomach and reduction to the ferrous form by compounds such as ascorbic acid or a ferri-reductase present at the musosal surfaces of cells in the duodenum. The ferrous bioavailable iron is then absorbed in a three-step process in which the iron is taken up by the enterocytes across the cellular apical membrane by an energy-dependent, carrier-mediated process, transported intracellularly, and transferred across the basolateral membrane into the plasma.

The size of the intracellular iron pool has a regulatory role in the synthesis of iron storage, iron transport, and iron metabolism proteins. Iron is stored as ferritin or hemosiderin. Hemosiderin is a water-insoluble degradation product of ferritin. The iron content of hemosiderin is variable but generally higher than that of ferritin. The cells of the liver, spleen, and bone marrow are the primary iron storage sites in humans. Only a small quantity of iron is lost each day as iron is highly conserved. The majority of absorbed iron is excreted in the feces. IOM states that the bioavailability of iron in the U.S. diet is estimated to be 18%.

Given the low anticipated exposure by an individual to iron from the intended use of the anti-caking agent in salt (0.16 – 0.26 mg/day; see Table in Question 1 Response) which represents a minimal contribution to dietary intakes of iron, along with its low bioavailability, there is no potential for exceeding the RDA or UL (see Tables below in reponse to Question 2) for iron as a result of the proposed use as an anti-caking agent in salt. Furthermore, it will not increase the body's iron burden in individuals with no iron deficiency and the risk of adverse effects related to iron consumption from the proposed use is negligible.

2. In the "Introduction" section of the "Safety" narrative on p 18, you state that "RDA for iron is 8 mg/day for all groups of men and postmenopausal women and 18 mg/day for premenopausal women. A UL of 40 mg/day for children (1–13 years old) and 45 mg/day adults (>14 years old) was established." The statement on the IOM tolerable upper intake level (UL) is stated again on p 24 ("Safety Summary" section) and p 26 ("GRAS of Iron Citrate" section).
   a. If you intend exposure to iron citrate, the subject of this notice, to include children and adolescents, please describe the IOM RDA values for different age, gender, and life stage groups in these subpopulations.

Response:

As an addendum to the RDAs for iron described in GRN 1036, the following tables describe RDAs and ULs for additional age, gender, and life stage groups.

Age	**Iron RDA (mg/day)***
1-3 years	7
4-8 years	10

Age	Iron RDA (mg/day)*
9-13 years (boys)	8
9-13 years (girls)	8
14-18 years (boys)	11
14-18 years (girls)	15
19-30 years (men)	8
19-30 years (women)	18
31-50 years (men)	8
31-50 years (women)	18
51-70 years (men)	8
51-70 years (women)	8
>70 years (men)	8
>70 years (women)	8
14-18 years (pregnancy)	27
19-30 years (pregnancy)	27
31-50 years (pregnancy)	27
14-18 years (lactation)	10
19-30 years (lactation)	9
31-50 years (lactation)	9

*IOM (2001)

Age	Iron UL (mg/day)*
0-12 months	40
1-3 years	40
4-8 years	40
9-13 years	40
14-18 years	45
Adults (≥19 years)	45
14-18 years (pregnancy)	45
19-50 years pregnancy)	45
14-18 years (lactation)	45
19-50 years (lactation)	45

*IOM (2001)

3. All the toxicity studies described in the "Animal Studies" section administered ferric citrate. In contrast, the proposed ingredient predominantly consisted of ferrous citrate. To allow for an appropriate comparison between animal toxicity studies please provide the following information.
   a. Please provide a summary table that contains the administered levels of exposure to ferric citrate for each study and the associated level of iron exposure (expressed as mg/kg bw/day) associated with study treatment levels.
   b. Please also include in the table the duration of exposure of the experimental treatment and the NOAEL and LOAEL values (expressed as mg iron/kg bw/day) identified for each study.
   c. Last, based on the information on iron exposure presented in the table, please discuss your stated conclusion in the notice (e.g., found in the summary sections of "Safety

Summary," "Safety Determination" and "GRAS of Iron Citrate") that the findings from these toxicity studies support the safety of the intended use of the iron citrate ingredient.

Response:

The following table provides a summary of the requested data and information.

Study	Ferric Citrate (mg/kg/day)	Iron (mg/kg/day)*	Study Duration	NOAEL or LOAEL (mg iron/kg/day)
Iida et al. (2020)	0.3 and 3% in the diet; equivalent to approx. 300 and 3000 mg/kg/day	63 and 630 mg/kg/day	11 days	LOAEL: 630 NOAEL: 63; for effects on phosphorus metabolism
Pallares et al. (1996)	22.5 mg/kg/day	4.5 mg/kg/day	40 days	NOAEL: 4.5 mg/kg/day
Yokoi et al. (2018)	0, 3.5, 7, 10.5, 14, 24.5, 63, 126 mg/kg/day	0, 0.7, 1.4, 2.1, 2.8, 4.9, 12.6, 25.2 mg/kg/day	5 weeks	Not stated; 4.9 – 12.6 mg/kg/day required to affect hemoglobin and red cell counts
Lau et al. (2018)	4% in the diet or approximately 4000 mg/kg/day	800 mg/kg/day	6 weeks	NOAEL: 800 mg/kg/day
Inai et al. (1994)	0.06, 0.12, 0.25, 0.5, 1% in drinking water of mice; estimated to be approx. 3, 6, 12, 24, and 48 mg/kg/day	approximately 0.6, 1.2, 2.4, 4.8, 9.6 mg/kg/day	13 weeks	MTD: 1.2 mg/kg/day NOAEL: 0.6 mg/kg/day
Toyoda et al. (2014)	Males: 0, 144, 596, 2835 Females: 0, 148, 601, 2846	Males: 0, 29, 119, 567 Females: 0, 30, 120, 569	13 weeks	NOAEL: 119 (males); 120 (females)
Luo et al. (2020)	83.3, 166.6, 333.3 mg/kg/day in mice	16.7, 33.3, 66.7	16 weeks	NOAEL: 33.3 mg/kg/day
Inai et al. (1984)	0.06 and 0.12% in drinking water of mice; 3.4 and 7.7 (males); 2.3 and 5.2 females)	0.7 and 1.5 (males); 0.5 and 1.0 females)	96 weeks	NOAEL: 1.5 (males); 1.0 (females)

*Assumes iron content of ferric citrate of approximately 21% when iron content of test article/diet is not specified.

The existing study information/data described above for ferric citrate addresses toxicological endpoints relevant to the human oral consumption of iron citrate (e.g., short-term and subchronic oral toxicity, chronic toxicity/carcinogenicity). Toxicological studies employing ferric citrate are considered relevant and extrapolatable to ferrous citrate (see answer to Question 4 below). The NOAELs for iron in the above studies are supportive of the ULs summarized in response to Question 2 above. Upper intake limits (ULs) of 40 mg/day for children (1–13 years old) and 45 mg/day adults (>14 years old) have been established for iron by the Food and Nutrition Board of the National Institute of Medicine, The potential iron intake resulting from iron citrate as an anti-caking agent in salt (0.16 - 0.26 mg/person/day) is well below the ULs. Therefore, it can be concluded that the publicly available data/information on iron citrate (ferric and ferrous forms) are sufficient to support the safe use of iron citrate for the proposed anti-caking use in salt.

4. Please discuss whether studies conducted with ferric-based and ferrous-based substances are comparable and the findings are extrapolatable in the safety evaluation of iron. You may include in your discussion the fate of dietary non-heme iron that undergoes several cycles of oxidation and reduction [Fe(II) ↔ Fe(III)] before reaching the storage form.

Studies conducted with ferric-based (Fe^{3+}) substances are comparable to ferrous-base (Fe^{2+}) substances and the findings can be extrapolated in the safety evaluation of iron citrate. This is because absorption of dietary iron occurs in the intestine and depends heavily on the physical state of the iron atom. At physiological pH, iron exists in the ferric state (Fe^{3+}), but to be absorbed iron must be in the ferrous state (Fe^{2+}) or bound be a protein, such as heme (Ems et al., 2022). Non-heme iron comes from food and is present mainly as ferric iron (Fe^{3+}) (Santiago, 2012). Therefore, it must be reduced to the ferrous and divalent form (Fe^{2+}) prior to absorption, allowing it to enter systemic circulation. Once absorbed and inside the cell (enterocytes), iron can be stored as ferritin or transported through the basolateral membrane and into circulation bound to ferroportin (Ems et al., 2022). Ferritin that is not bound to iron is called apoferritin, which has an intrinsic catalytic activity that oxidizes ferrous iron (Fe^{2+}) into ferric iron (Fe^{3+}) to be bound and stored as ferritin (Ems et al., 2022). These cycles of oxidation and reduction play a critical role in iron absorption and homeostasis in the body as it is an essential cofactor required for the activity of many essential enzymes and molecules (Wallace, 2016). Taken together, safety studies using ferric iron (Fe^{3+}) can be extrapolated for use in the safety evaluation of ferrous iron (Fe^{2+}).

Ems T, St Lucia K, Huecker MR. 2022. Biochemistry, Iron Absorption. StatPearls. https://www.ncbi.nlm.nih.gov/books/NBK448204/. Accessed 05/26/22.

Wallace DF. 2016. The Regulation of Iron Absorption and Homeostasis. Clin Biochem Rev. 37(2):51-62. PMID: 28303071; PMCID: PMC5198508.

Santiago P. 2012. Ferrous versus ferric oral iron formulations for the treatment of iron deficiency: a clinical overview. ScientificWorldJournal. 2012:846824. doi: 10.1100/2012/846824.

5. On p 24, you describe a study that administered ferrous citrate mixed in course crude cooking salt to humans.
   a. Please indicate the exposure to ferrous citrate expressed as mg/kg bw and indicate the frequency or time period over which the agent was administered.
   b. Also please indicate how long the study subjects were observed post-treatment.
   c. Last, from the ferrous citrate dose administered, please provide the exposure to iron as mg iron/kg bw.

Response:

There is an error in the study description on p.24. Ferrous citrate was not administered to the human subjects in course crude cooking salt, but rather as radiolabelled ferrous citrate for a determination of iron absorption.

a. The ferrous citrate test article was administered as a single dose. The authors state that the ferrous citrate test article was given to one group of male and female subjects at 3 mg iron after an overnight fast and to a second group of male and female subjects at 7.5 mg iron with a meal. Body weights of the male and female subjects were not stated, but given a body weight of 60 kg, the exposure to iron from the iron citrate test article was 0.05 mg iron/kg bw for the fasted group and 0.125 mg iron/kg bw for the group provided a meal at the time of consumption. Employing an estimate of iron accounting for approximately 20% of the ferrous citrate test article, then the estimated exposure to ferrous citrate was 0.25 mg/kg bw for the fasted group and 0.625 mg/kg bw for the group provided a meal with the test article.
b. The length of time the study subjects were observed post-treatment was not stated by the authors. However, the authors stated that all subjects had standard ferrous ascorbate absorption determined 12 days after the ferrous citrate absorption study dose.
c. The ferrous citrate test article was given to one group of subjects at 3 mg iron after an overnight fast and to a second group at 7.5 mg iron with a meal. Body weights of the male and female subjects were not stated, but given a body weight of 60 kg, the exposure to iron from the ferrous citrate test article was 0.05 mg/kg bw for the fasted group and 0.125 mg/kg bw for the group provided a meal at the time of consumption.