29 May 2020

Dr. Paulette Gaynor
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration
5001 Campus Drive
College Park, MD
20740 USA

Dear Dr. Gaynor:

Re: GRAS Notice for Iron Milk Proteinate

In accordance with 21 CFR §170 Subpart E consisting of §§ 170.203 through 170.285, Société des Produits Nestlé S.A. [Avenue Nestlé 55, CH-1800, Vevey, Switzerland], as the notifier, is submitting one hard copy and one electronic copy (on CD), of all data and information supporting the company's conclusion that iron milk proteinate, is GRAS on the basis of scientific procedures, for use as a dietary source of iron in conventional food and beverage products; these food uses of iron milk proteinate are therefore not subject to the premarket approval requirements of the *Federal Food, Drug and Cosmetic Act*. Information setting forth the basis for Nestlé's GRAS conclusion, as well as a consensus opinion of an independent panel of experts, also are enclosed for review by the agency.

I certify that the enclosed electronic files were scanned for viruses prior to submission and are thus certified as being virus-free using Symantec Endpoint Protection 12.1.5.

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,

Michel Donat
Global Head Regulatory & Scientific Affairs
Dairy Strategic Business Unit
Société des Produits Nestlé S.A.
Avenue Nestlé 55
1800 Vevey
Switzerland

Email: Michel.Donat@rdls.nestle.com
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GRAS NOTICE FOR THE USE OF IRON MILK PROTEINATE IN CONVENTIONAL FOOD AND BEVERAGE PRODUCTS

SUBMITTED TO:
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration
5001 Campus Drive
College Park, MD
20740 USA

SUBMITTED BY:
Société des Produits Nestlé S.A.
Avenue Nestlé 55
1800 Vevey
Switzerland

DATE:
29 May 2020

GRAS Notice for the Use of Iron Milk Proteinate in Conventional Food and Beverage Products

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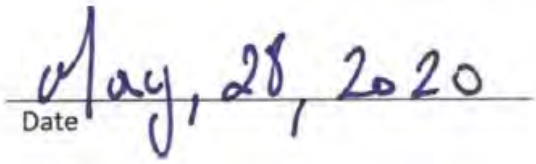
GRAS Notice for the Use of Iron Milk Proteinate in Conventional Food and Beverage Products

PART 1. § 170.225 Signed Statements and Certification

In accordance with 21 CFR §170 Subpart E consisting of §§170.203 through 170.285, Société des Produits Nestlé S.A. (Nestlé) hereby informs the United States (U.S.) Food and Drug Administration (FDA) that iron milk proteinate (IMP) is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on Nestlé's view that IMP is Generally Recognized as Safe (GRAS). In addition, as a responsible official of Nestlé, the undersigned hereby certifies that all data and information presented in this notice represent a complete and balanced submission that is representative of the generally available literature. Nestlé considered all unfavorable, as well as favorable, information that is publicly available and/or known to Nestlé and that is pertinent to the evaluation of the safety and GRAS status of IMP as a dietary source of iron in conventional food and beverage products as described herein.

Signed,

Michel Donat
Global Head Regulatory & Scientific Affairs
Société des Produits Nestlé S.A.
Michel.Donat@rdls.nestle.com



Michel Donat
Global Head Regulatory & Scientific Affairs
Société des Produits Nestlé S.A.
Michel.Donat@rdls.nestle.com

1.1 Name and Address of Notifier

Société des Produits Nestlé S.A.
Avenue Nestlé 55
1800 Vevey
Switzerland
Tel: +41 21 924 61 89

1.2 Common Name of Notified Substance

Iron milk proteinate; IMP; iron-casein-phosphate complex; ferric caseinate

1.3 Conditions of Use

Iron milk proteinate is intended for use as a dietary source of iron in conventional food and beverage products in the U.S., and its uses will be fully substitutional to other iron ingredients currently on the U.S. marketplace. Iron milk proteinate will be used in the same food categories as other iron salts at levels based on current Good Manufacturing Practice (cGMP) in accordance with the principles of the U.S. FDA's fortification guidelines and Fortification Policy under 21 CFR §104.20 (U.S. FDA, 2019). As an example, a serving of food to which iron milk proteinate may be added is anticipated to contain in the region of 244 mg of iron milk proteinate providing *ca*. 6 mg of ferric iron. As all food uses of iron milk proteinate will be

substitutional to current sources of iron used for food fortification, the intended conditions of use of iron milk proteinate will not change the current dietary intakes of iron in the U.S. population.

1.4 Basis for GRAS

Pursuant to 21 CFR § 170.30 (a)(b) of the Code of Federal Regulations (CFR) (U.S. FDA, 2019), Nestlé has concluded that the intended uses of iron milk proteinate, as described herein, are GRAS on the basis of scientific procedures.

1.5 Availability of Information

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

Société des Produits Nestlé S.A.
Avenue Nestlé 55
1800 Vevey
Switzerland

Should the U.S. FDA have any questions or additional information requests regarding this Notification, Nestlé will supply these data and information upon request.

1.6 Freedom of Information Act, 5 U.S.C. 552

It is Nestlé's view that all data and information presented in Parts 2 through 7 of this Notice do not contain any trade secret, commercial, or financial information that is privileged or confidential, and therefore, all data and information presented herein are not exempted from the Freedom of Information Act, 5 U.S.C. 552.

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

2.1 Identity of the Ingredient

Iron milk proteinate is a complex of ferric iron (Fe^{3+}) bound to sodium caseinate in the presence of orthophosphate (Figure 2.1-1). Phosphoserine residues of casein are the primary binding sites of ferric ions stabilized by inorganic phosphate. The complex has an iron loading of at least 2% (w/w). Iron milk proteinate is a dark-red-to-orange powder that is highly soluble in water. The molecular weight of iron milk proteinate was determined to be in the range of $1x10^8$ to $3x10^8$ Da using size exclusion chromatography coupled with multi-angle laser light scattering. The physical and chemical structures of iron milk proteinate was characterized by transmission electron microscopy combined with energy dispersive X-ray scattering (TEM-EDS), which shows the presence of carbon, nitrogen, oxygen, iron, and phosphorus (Figure 2.1-2).

Figure 2.1-1 Schematic of the Structure of Iron Milk Proteinate

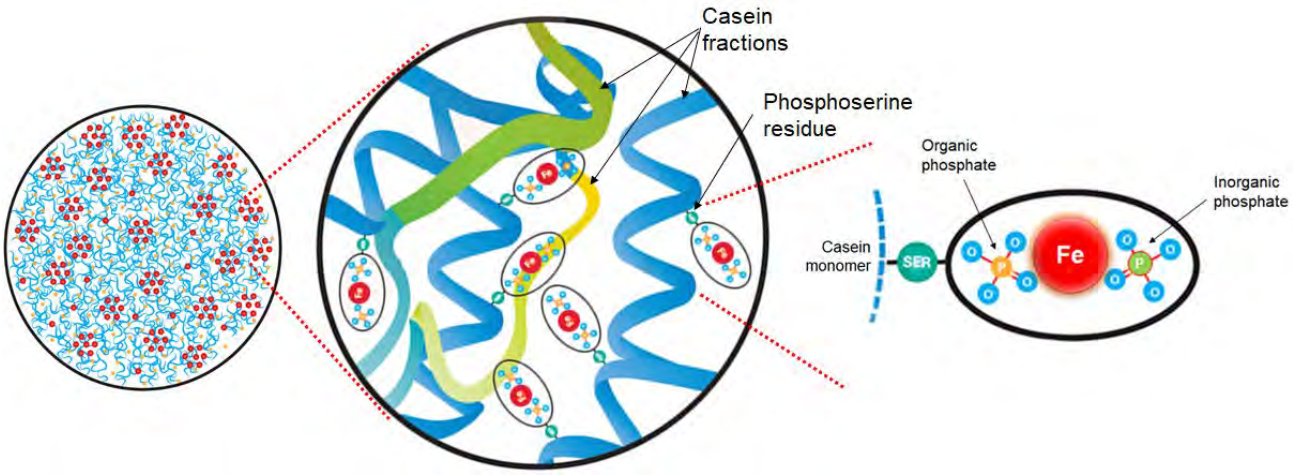
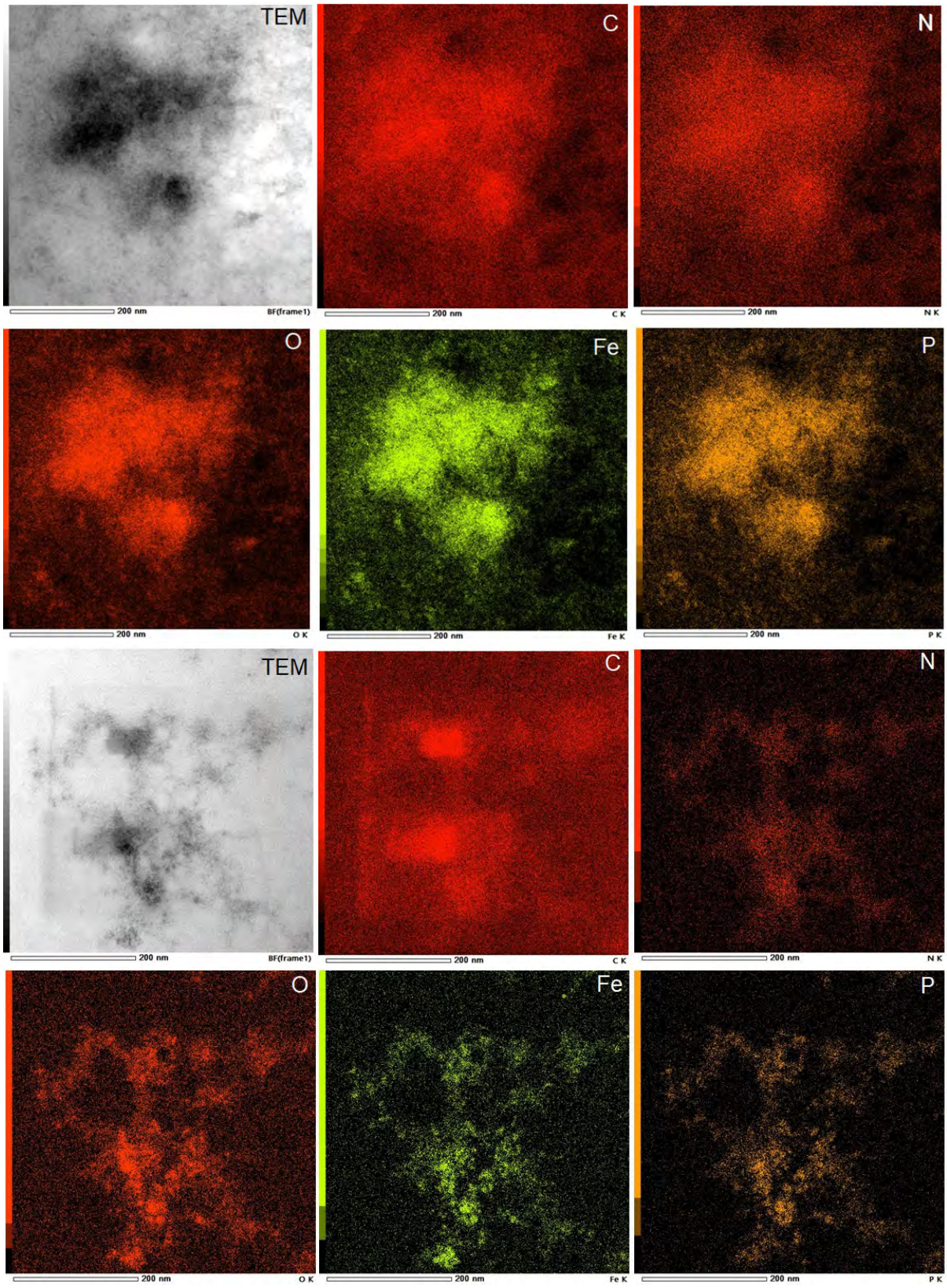


Figure 2.1-2 Chemical Structure of Iron Milk Proteinate by Transmission Electron Microscopy with Energy Dispersive X-Ray Scattering



2.2 METHOD OF MANUFACTURE

Iron milk proteinate is manufactured in a production facility certified under FSSC 22000 and complies with the principles of Hazard Analysis and Critical Control Point (HACCP). A schematic overview of the manufacturing process for iron milk proteinate is provided in Figure 2.2-1 below. All raw materials, processing aids, and purification aids used in the manufacturing process are food-grade or equivalent (*e.g.*, Food Chemicals Codex, United States Pharmacopeia, or European Pharmacopeia), and are used in accordance with an applicable FDA regulation, have previously been determined to be GRAS for their intended use, or have been the subject of an effective food contact notification.

Iron milk proteinate is produced by the addition of ferric salt[1] to a solution of casein[2] in the presence of orthophosphate. In the first step, deionized water is heated and sodium caseinate powder is slowly added until it is completely dissolved. The dissolved caseinate solution is then cooled to ambient temperature, and dipotassium hydrogen orthophosphate solution is added. Next, a solution of ferric salt is added slowly to avoid precipitation of the ions. The pH of the solution is maintained at roughly neutral pH with food-grade alkaline solution[3] to ensure that the iron ions do not precipitate from the solution. The solution is then vigorously stirred for 10 minutes followed by pasteurization, and then concentrated to a total solids level of up to 20% w/w. The solution may be spray-dried, yielding the final iron milk proteinate powder, or maintained as an iron milk proteinate solution.

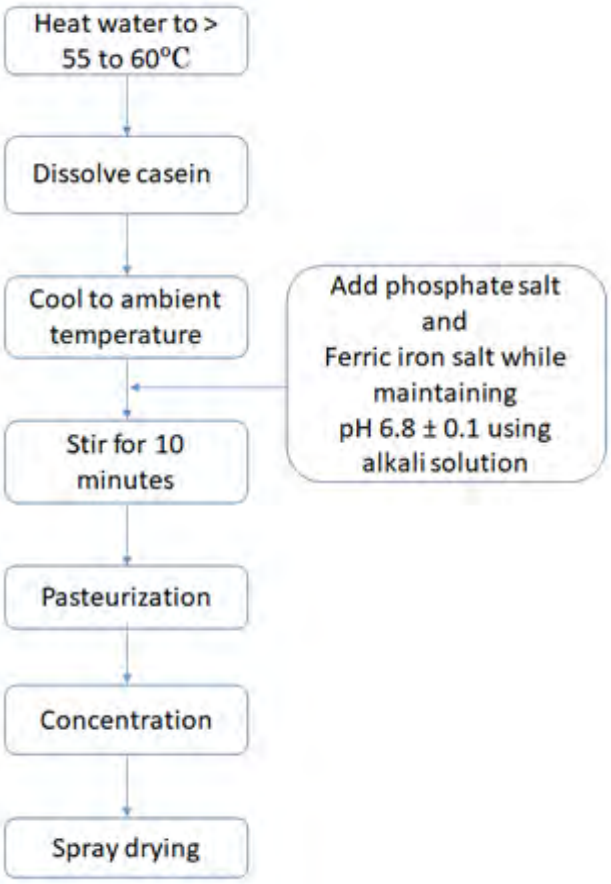
The production process does not involve any physical processing steps such as grinding, micronization, or milling that would micronize the final product. Particle size analysis on the final reconstituted powdered product demonstrates the average particle size to be in the range of 100 nm to 1 µm. As demonstrated in Sections 2.4 and 2.5, analytical data on potential impurities that may be introduced from the manufacturing process confirm the absence of any toxicological or microbiological hazards arising from the production of iron milk proteinate that would have an adverse effect on health.

[1] Ferric salt may also be added to the solution of casein or its derivatives before the addition of dipotassium hydrogen orthophosphate. The ferric ions bind strongly to the organic phosphorous on caseins *via* coordination bonds, forming a casein-iron precipitate that is darker in colour. The precipitate forms as a slurry, and following the addition of dipotassium hydrogen orthophosphate, a visibly reduced viscosity is observed. The sequence of ferric salt addition does not result in any differences in the composition of the final product as shown in Section 2.4.

[2] Nestle certifies that casein ingredients used for the production iron milk proteinate will meet the relevant U.S. standards and regulations for milk and milk products concerning current Good Manufacturing Practices (cGMP; 21 CFR §110); mandatory pasteurization requirements (21 CFR §1240.61); the U.S. standards on veterinary drugs (safe level and tolerance): Grade A Pasteurized Milk Ordinance; the U.S. standard on pesticides (40 CFR Part 180 and CPG §560.750); and the tolerances for polychlorinated biphenyls (PCBs) as laid down by 21 CFR §109.30 (1.5 ppm fat basis) (U.S. EPA, 2019; U.S. FDA, 2005, 2017, 2019).

[3] Alkaline solution examples include sodium hydroxide or potassium hydroxide or other food-grade alkaline solutions that are permitted for use in the U.S.

Figure 2.2-1 Schematic Overview of the Manufacturing Process for Iron Milk Proteinate



2.3 Product Specifications

Food-grade specifications have been established for iron milk proteinate (Table 2.3-1). All methods of analysis are internationally recognized [*e.g.,* Association of Official Analytical Chemists (AOAC)] or have been developed internally by Nestlé. Iron milk proteinate is generated from the reaction of ferric salt, casein or casein derivatives, and phosphate salt, resulting in complexation of iron-casein-phosphate. The ingredient is comprised primarily of protein (≥50%) and ash (≥27%) and contains at least 2% iron and 3% phosphorous.

Table 2.3-1 Product Specifications for Iron Milk Proteinate

Parameter	Specification	Method of Analysis
Appearance	White to off-white powder	Visual
Odor	Odorless	Organoleptic
Taste	Neutral	Organoleptic
Proximate Parameters		
Moisture	<8%	AOAC 945.15
Fat	<1%	AOAC 948.15
Total protein (as is)	≥50%	AOAC 992.15
Ash	≥27%	AOAC 942.05
pH (1% solution)	6.5 to 7.2	pH meter
Lactose	<0.1%	Internal method
Chemical Parameters		
Calcium	<0.13%	ICP-OES
Sodium	<5%	ICP-OES
Chloride	<0.1%	AOAC (OMA) 971.27, 976.18
Phosphorous	≥3%	ICP-OES
Iron	≥2%	ICP-OES
Heavy Metals		
Lead	<0.5 mg/kg	ICP-MS
Arsenic	<1 mg/kg	ICP-MS
Cadmium	<0.5 mg/kg	ICP-MS
Microbiological Parameters		
Aerobic plate count	<150 CFU/g	AOAC Research Institute Certification Number 121204[a]
Yeasts and molds	<10 CFU/g	AOAC Research Institute Certification Number 041001[b]
Coliforms	<10 CFU/g	AOAC Research Institute Certification Number 060702[c]
Escherichia coli	Negative in 25 g	AOAC Research Institute Certification Number 080603[d]
Salmonella spp.	Negative in 25 g	qPCR[e]
Staphylococcus aureus	Negative in 1 g	APHA 39 modified[f]

AC = aerobic count; AOAC = Association of Official Analytical Chemists; APHA = American Public Health Association; CC = coliforms count; CFU = colony-forming units; ICP-MS = inductively coupled plasma-mass spectrometry; ICP-OES = inductively coupled plasma-optical emission spectrometry; LT = lauryl tryptose; MPN = most probable number; qPCR = qualitative real-time polymerase chain reaction; TBX = tryptone bile glucuronide medium; TSB = tryptic soy broth; YM = yeast/mold.

[a] Automated MPN count on TEMPO AC, incubated at 30°C for 22 to 28 hours.

[b] Automated MPN count on TEMPO YM, incubated at 25°C for 72 to 76 hours.

[c] Automated MPN count on TEMPO CC, incubated at 35°C for 22 to 27 hours.

[d] LT broth at 35°C for 48 hours, TBX confirmation at 44°C for 24 hours (APHA 9.93 modified).

[e] IANZ and RLP-accredited methods.

[f] TSB at 35 to 37°C for 3 hours, TSB 10% salt at 35 to 37°C for 24 hours, Baird Parker agar.

2.4 Product Analysis of Iron Milk Proteinate

2.4.1 Proximate Analyses

Analysis of 5 non-consecutive lots of iron milk proteinate demonstrates that the manufacturing process as described in Section 2.2 produces a consistent product that meets the established product specifications. A summary of the analysis for the 5 lots of iron milk proteinate is presented in Table 2.4.1-1.

Table 2.4.1-1 Proximate Analyses of 5 Non-Consecutive Lots of Iron Milk Proteinate

Specification Parameter	Specification Limit	Manufacturing Lot					AVG	±	SD
		742344	742372	742376	742382	742386			
Moisture	<8%	2.9	2.6	3.2	3.3	3.3	3.1	±	0.27
Fat	<1%	0.6	0.4	0.3	0.2	<0.1	0.38	±	0.15
Total protein (as is)	≥50%	61	55	63	63	62	61	±	2.99
Ash	≥27%	32	39	31	31	31	33	±	3.12
Lactose monohydrate	<0.1%	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1		
Calcium	<0.13%	0.05	0.04	0.05	0.04	0.05	0.05	±	0.00
Sodium	<5%	2.3	2.7	2.2	2.0	2.2	2.3	±	0.23
Phosphorous	≥3%	3.5	4.0	3.5	3.1	3.5	3.5	±	0.29
Chloride	<0.1%	0.08	0.08	0.08	0.08	0.08	0.08	±	0.00
Iron	≥2%	2.7	3.4	2.7	2.3	2.6	2.7	±	0.36
pH (1% solution)	6.5 to 7.2	6.9	6.8	6.9	6.9	6.9	6.9	±	0.04

AVG = average; SD = standard deviation.

2.4.2 Microbiological Analysis

Analysis of 5 non-consecutive lots of iron milk proteinate demonstrates that the product that meets the microbiological specifications outlined in Section 2.3. A summary of the microbiological analysis for the 5 lots of iron milk proteinate is presented in Table 2.4.2-1.

Table 2.4.2-1 Microbiological Analyses of 5 Non-Consecutive Lots of Iron Milk Proteinate

Specification Parameter	Specification Limit	Manufacturing Lot					AVG	±	SD
		742344	742372	742376	742382	742386			
Aerobic plate count (30°C)	<10,000 CFU/g	110	89	21	<10	10	40	±	35
Yeast and molds	<10 CFU/g	<10	<10	<10	<10	<10	<10		
Total coliforms	<10 CFU/g	<10	<10	<10	<10	<10	<10		
Escherichia coli	<10 CFU/g	ND	ND	ND	ND	ND	ND		
Salmonella spp.	Negative/25 g	ND	ND	ND	ND	ND	ND		
Staphylococcus aureus	Negative/g	ND	ND	ND	ND	ND	ND		

CFU = colony forming units; ND = not detected.

2.5 Additional Chemical Characterization

2.5.1 Heavy Metals and Minerals

Six non-consecutive lots of iron milk proteinate were analyzed for heavy metals. The results of the analysis are summarized in Table 2.5.1-1. All analyses were conducted using inductively coupled plasma mass spectrometry (ICP-MS).

Table 2.5.1-1 Heavy Metal and Minerals Analyses of 6 Non-Consecutive Lots of Iron Milk Proteinate

Specification Parameter	Specification Limit	Manufacturing Lot					
		17 FP201	19 FP202	23 FP203	18 FP301	20 FP302	24 FP303
Antimony (mg/kg)	N/A	<2[a]	<2	<2	<2	<2	<2

Table 2.5.1-1 Heavy Metal and Minerals Analyses of 6 Non-Consecutive Lots of Iron Milk Proteinate

Specification Parameter	Specification Limit	Manufacturing Lot					
		17 FP201	19 FP202	23 FP203	18 FP301	20 FP302	24 FP303
Arsenic (mg/kg)	<1	<0.01	<0.01	<0.01	<0.01	<0.01	0.5
Bismuth (mg/kg)	N/A	<0.2[b]	<0.2	<0.2	<0.2	<0.2	<0.2
Cadmium (mg/kg)	<0.5	<0.08	<0.08	<0.08	<0.08	<0.08	<0.08
Copper (mg/kg)	N/A	3.3	2.9	3.0	2.6	2.7	6.5
Lead (mg/kg)	<0.5	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
Silver (mg/kg)	N/A	<0.2[c]	<0.2	<0.2	<0.2	<0.2	<0.2
Tin (mg/kg)	N/A	<1.0[d]	<1.0	<1.0	<1.0	<1.0	1.0

ICP-MS = inductively coupled plasma-mass spectrometry; N/A = not available.
[a] Limit of detection = 2 mg/kg
[b] Limit of detection = 0.2 mg/kg
[c] Limit of detection = 0.2 mg/kg
[d] Limit of detection = 1 mg/kg

2.6 Stability of Iron Milk Proteinate

2.6.1 Storage Stability

The shelf-life storage stability of iron milk proteinate was investigated with 2 lots of iron milk proteinate (Lot Nos. 19FP204 and 25FPS201). Samples were kept in foil laminate that is resistant to light, moisture, and air at 20°C for up to 12 months and at 37°C for up to 12 months. The proximate composition of iron milk proteinate is not expected to change during storage and hence the samples from the storage study were investigated for their characteristics such as their color, solubility, and particle sizes upon reconstitution and release of any free iron during storage. The study is currently on going and data is available up to 6 months. The results indicate no change in color, solubility, or particle size when the iron milk proteinate is stored for up to 6 months at 20 or 37°C. In addition, no changes in the ratio of iron, protein, and phosphorus content of the iron milk proteinate were reported.

Table 2.6.1-1 Proximate Composition of Iron Milk Proteinate Used in the Storage Study

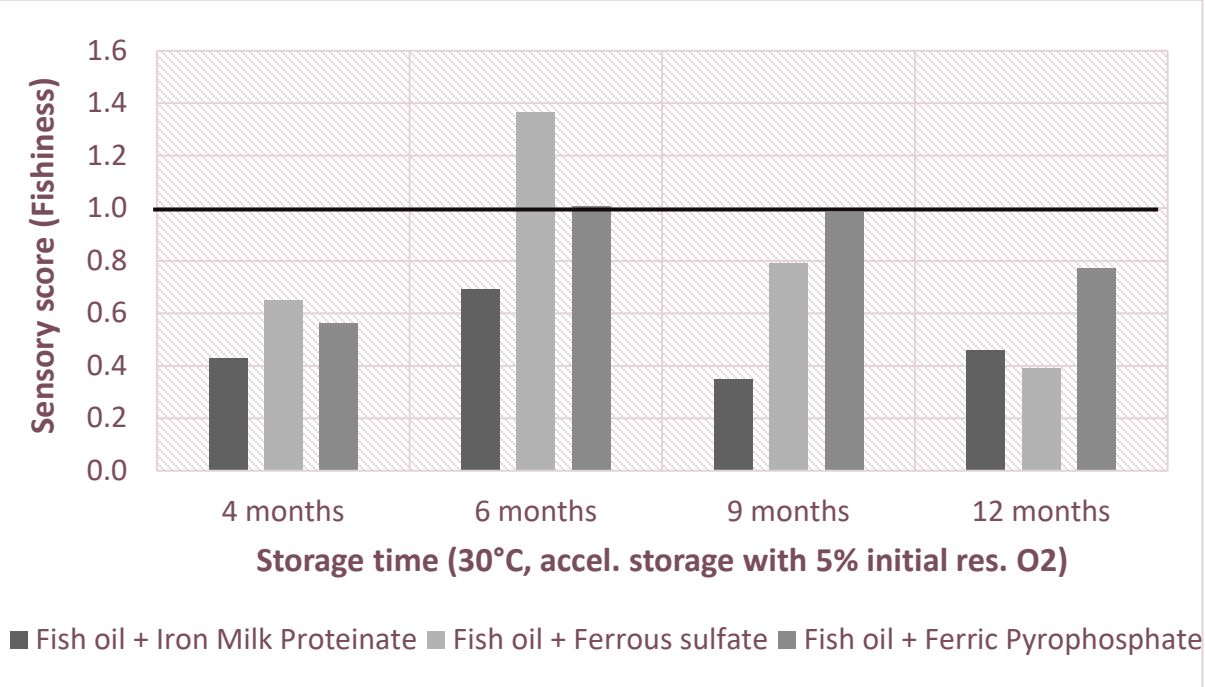
Parameter	Batch No.	
	B1 (19FP204)	B2 (25FPS201)
Fat (%)	0.5	0.8
Protein (%)	65	66
Ash (%)	28	26
Total Solids (%)	94	95
Iron (%)	2.6	2.2
Phosphorous (%)	3.6	3.8

2.6.2 Sensory Stability

The sensory stability of iron milk proteinate was investigated in 2 studies in which the complex was added in milk powder containing fish oil and in a non-dairy ready-to-drink (RTD) beverage. In the first study, iron milk proteinate in skimmed milk powder containing high docosahexaenoic acid (DHA) fish oil (0.3% DHA) and iron (0.015%) was stored in 200 mL aluminum cans with an initial residual oxygen content of 5% at 30°C for up to 12 months. Ferrous sulfate and ferric pyrophosphate were also mixed in a similar manner for comparative purposes. As shown in Figure 2.6.2-1 below, the sensory score (for “fishiness”) of the iron milk

proteinate was consistently less than 1.0 over the 12-month storage period. In comparison, the sensory score of the ferrous sulfate mixture exceeded 1.0 at 6 months, while the sensory score for the ferric pyrophosphate mixture was around 1.0 at 6 and 9 months of storage. A sensory score of 1.0 is considered the consumer acceptance limit with respect to "fishiness" of the product.

Figure 2.6.2-1 Changes in Sensory Profile of Milk Powder Containing Fish Oil and Iron Milk Proteinate, Ferrous Sulfate, or Ferric Pyrophosphate over 12 months (30°C, 5% initial residual oxygen content)



Note: The black horizontal line represents consumer acceptance limit.

In the second study, iron milk proteinate or ferric pyrophosphate was added to a non-dairy RTD beverage containing DHA oil and stored at 30°C for 9 months. A trained sensory panel consumed the beverage after 3, 5, 7, and 9 months of storage and evaluated the taste of the beverage based on fishiness, rancidity, and metallic. No significant differences in these sensory parameters were reported between the 2 sources of iron. Findings from this study demonstrate complexation of iron within iron milk proteinate preventing iron from oxidizing polyunsaturated fatty acids within food matrices and therefore will not produce undesirable off-flavors in food products rich in unsaturated fats.

2.7 Technical Effect

Iron milk proteinate is intended for use as a source of dietary iron and will be fully substitutional to other iron ingredients that are permitted for addition to food by regulation or that have GRAS status for specified food uses in the U.S. marketplace. Iron milk proteinate is a dark-red to orange powder that is highly soluble in water, and produces a comparable iron bioavailability to ferrous sulfate (see Section 6.3.1 for further details) without compromising the organoleptic properties of the finished foods to which it is added. As discussed in Section 2.6.3, incorporation of iron milk proteinate into different food matrices does not result in any changes in the sensory profile with respect to taste and rancidity.

PART 3. § 170.235 DIETARY EXPOSURE

3.1 Current Regulatory Status

3.1.1 Iron

A number of iron compounds were affirmed as GRAS by the U.S. FDA for use as direct food substances with no limitation other than cGMP under 21 CFR §184 (U.S. FDA, 2019) and 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. In addition, multiple iron ingredients have been concluded to be GRAS for specified food uses and have been notified to the U.S. FDA and filed without objection (Table 3.1.1-1).

Table 3.1.1-1 Summary of GRAS Notifications for Substances Providing a Source of Dietary Iron

GRN No.	Substance	Intended Use	Reference
19	Ferrous bisglycinate chelate	Use in foods in general as a source of dietary iron for food enrichment and fortification purposes consistent with iron supplementation guidelines.	U.S. FDA (1999)
152	Sodium iron EDTA	Use in iron fortification of powdered soft drinks in areas of the world with a high prevalence of iron deficiency at a level of 2.5 mg iron/200 mL of reconstituted beverage.	U.S. FDA (2004a)
178	Sodium iron EDTA	Iron fortification of soy, fish, hoisin and teriyaki sauces at an iron level of 0.024%; and of sweet and sour sauce at an iron level of 0.012%.	U.S. FDA (2006)
271	Ferrous ammonium phosphate	Use in various food categories as a source of dietary iron for food enrichment and fortification purposes consistent with iron supplementation guidelines.	U.S. FDA (2009)
441	Sodium ferrous citrate	Use in various food categories as a source of dietary iron for food enrichment and fortification purposes consistent with iron supplementation guidelines.	U.S. FDA (2013)

EDTA = ethylenediaminetetraacetic acid; GRN = GRAS Notice; U.S. = United States.

The Institute of Medicine (IOM) has established estimated average requirements (EAR), recommended daily allowances (RDA) and tolerable upper limits (UL) for iron for different population groups (*e.g.,* children, adults) (IOM, 2001). The RDAs and UL for the different population groups are summarized in Table 3.1.1-2. The FDA has established reference daily intake (RDI) or daily value (DV) of 18 mg/person/day for iron under 21 CFR §101.9 (U.S. FDA, 2019). Iron containing ingredients are typically added to food as a percentage of the RDI or DV to supplement iron intake from the natural food sources (*e.g*., meat and vegetables).

Current practices for adding iron to the diet typically consider the general labeling principles that a "good source" contains between 10% and 19% of the DV in the Reference Amounts Customarily Consumed (RACC) and an "excellent source" contains 20% of the DV or greater in the RACC (21 CFR §101.13) (U.S. FDA, 2019). The fortification of foods with nutrients such as iron must comply with the Fortification Policy (21 CFR §104.20) outlined in the FDA's Nutritional Quality Guidelines for Foods (21 CFR §104.20) (U.S. FDA, 2019). Importantly, the FDA's Fortification Policy specifically states that, "*A nutrient added to a food is appropriate only when the nutrient: Is present at a level at which there is a reasonable assurance that consumption of the food containing the added nutrient will not result in an excessive intake of the nutrient, considering cumulative amounts from other sources in the diet*" (21 CFR §104.20) (U.S. FDA, 2019). In addition, "*The Food and Drug Administration does not encourage indiscriminate addition of nutrients to foods, nor does it*

consider it appropriate to fortify fresh produce; meat, poultry, or fish products; sugars; or snack foods such as candies and carbonated beverages" (21 CFR §104.20) (U.S. FDA, 2019).

Table 3.1.1-2 Estimated Average Requirement, Recommended Daily Allowances and Tolerable Upper limits for Iron Established by the Institute of Medicine

Population Group	EAR (mg/day)	RDA (mg/day)	UL (mg/day)
Children			
1 to 3 years	3	7	40
4 to 8 years	4.1	10	40
Males			
9 to 13 years	5.9	8	40
14 to 18 years	7.7	11	45
19 to 30 years	6.0	8	45
31 to 50 years	6.0	8	45
51 to 70 years	6.0	8	45
>70 years	6.0	8	45
Females			
9 to 13 years	5.7	8	40
14 to 18 years	7.9	15	45
19 to 30 years	8.1	18	45
31 to 50 years	8.1	18	45
51 to 70 years	5.0	8	45
>70 years	5.0	8	45
Pregnancy			
≤18 years	7.0	27	45
19 to 50 years	6.5		
Lactation			
14 to 18 years	23	10	45
19 to 50 years	22	9	45

RDA = recommended daily allowance; UL = tolerable upper limit.

3.1.2 Phosphorus

The IOM noted that phosphorus is ubiquitous in the human diet as nearly all foods contain phosphorus and it is a common food additive (IOM, 2006). Phosphorus is present in the diet as phosphate or its various salts. The average adult diet contains approximately 62 mg phosphorus per 100 kcal. The IOM has established EAR, RDA, and UL values for the same population group as those for iron. These values are summarized in Table 3.1.2-1 below.

Table 3.1.2-1 Estimated Average Requirement, Recommended Daily Allowances and Tolerable Upper limits for Phosphorus Established by the Institute of Medicine

Population Group	EAR (mg/day)	RDA (mg/day)	UL (mg/day)
Children			
1 to 3 years	380	460	3,000
4 to 8 years	405	500	3,000
Males			
9 to 13 years	1,055	1,250	4,000
14 to 18 years	1,055	1,250	4,000
19 to 30 years	580	700	4,000
31 to 50 years	580	700	4,000
51 to 70 years	580	700	4,000
>70 years	580	700	3,000
Females			
9 to 13 years	1,055	1,250	4,000
14 to 18 years	1,055	1,250	4,000
19 to 30 years	580	700	4,000
31 to 50 years	580	700	4,000
51 to 70 years	580	700	4,000
>70 years	580	700	3,000
Pregnancy			
≤18 years	1,055	1,250	3,500
19 to 50 years	580	700	3,500
Lactation			
14 to 18 years	1,055	1,250	4,000
19 to 50 years	580	700	4,000

RDA = recommended daily allowance; UL = tolerable upper limit.

3.1.3 Casein

Casein and caseinates (including sodium and calcium salts) have an extensive history of safe consumption as they are naturally occurring milk proteins. Milk contains approximately 3% protein, of which 80% is casein while the remaining 20% is whey protein (Wattiaux, 1995). Sodium caseinate is GRAS for multiple purposes when used in accordance with Good Manufacturing Practice (GMP) under 21 CFR §182.1748 (U.S. FDA, 2019). The safety of casein and sodium caseinate and calcium caseinate was reviewed by the FDA Select Committee on GRAS Substances (SCOGS) and it was concluded that there is no evidence to suggest "*reasonable grounds to suspect a hazard when they are used at levels that are now current or that may reasonably be expected in the future*", as these compounds are a major component of the human diet and have been consumed for centuries (FASEB, 1979) (see Section 6.4.2 for further details).

3.2 Functionality

Iron deficiency is the most common known form of nutritional deficiency in the U.S. (CDC, 1998). The prevalence of iron deficiency anemia is highest in young children and women of childbearing age (CDC, 1998; USDA, 2015). The USDA reported that iron is under consumed by adolescent and premenopausal females, including those that are pregnant; 96% of pregnant women had iron intakes below the EAR of 6.5 to 7.0 mg/day (USDA, 2015).

Iron milk proteinate is intended for use as a dietary source of iron. Fortification with iron will be in accordance with the Fortification Policy as discussed in Section 3.1. Iron milk proteinate has been demonstrated to have a similar bioavailability of iron compared to ferrous sulfate (see Section 6.3.1 for further details). The fortification of foods with iron compounds is generally based on achieving the maximum bioavailability, while not compromising the organoleptic properties of the finished foods to which they are added. As discussed in Section 2.6.3, incorporation of iron milk proteinate into different food matrices does not result in any changes in the sensory profile with respect to taste and rancidity.

3.3 Background Intakes of Iron and Phosphorus

Iron intake from the background diet is a result of consumption of foods naturally containing iron and foods supplemented with iron to help meet the RDI or DV of 18 mg/person/day for iron established by the FDA under 21 CFR §101.9 (U.S. FDA, 2019). As indicated in Section 3.1.1, 13 iron compounds are affirmed as GRAS by the U.S. FDA for use as direct food substances with no limitation other than cGMP under 21 CFR §184 (U.S. FDA, 2019). In addition, multiple iron ingredients have been concluded to be GRAS for use in specific foods for nutritional purposes and have been notified to the U.S. FDA and filed without objection (summarized in Table 3.1.1-1).

The IOM has established RDAs and ULs for iron and phosphorous for different population groups, including children 1 to 8 years, males and females 9 to >70 years, and pregnant and lactating women 14 to 50 years, presented in Tables 3.3-1 and 3.3-2, respectively (IOM, 2006). The IOM has also estimated usual intakes of iron and phosphorus at the mean and various percentiles for these same population groups using food consumption data that is representative of the U.S. population (IOM, 1997, 2001). Usual intakes of iron were estimated from food and supplements using food consumption data from the Third National Health and Nutrition Examination Survey (NHANES III, 1998-1994) and are presented in Table 3.3-1 (IOM, 2001), whereas usual intakes of phosphorous were estimated using food consumption data from the USDA Continuing Survey of Food Intake of Individuals (CSFII, 1994; adjusted for day-to-day variation according to Nusser *et al*., 1996) and are presented in Table 3.3-2 (IOM, 1997).

The estimated daily intake of iron from food and supplements at the mean and 90th percentile is above the RDA established for iron in all population groups except in females 14 to 50 years whose mean intakes were slightly below the RDA. In all population groups other than pregnant and lactating women, iron intakes at the mean and 90th percentile are 2.1 to 3.9 and 1.3 to 2.3 times lower than the UL established for iron, respectively (Table 3.3-1).

The estimated daily intake of phosphorous at the mean and 90th percentile is above the RDA established for phosphorous in all population groups except in females 9 to 18 years whose mean intakes were slightly below the RDA. In all population groups including pregnant and lactating women, phosphorous intakes at the mean and 90th percentile are 2.2 to 4.1 and 1.7 to 3.0 times lower than the UL established for phosphorous, respectively (Table 3.3-2).

Table 3.3-1 Recommended Daily Allowances and Tolerable Upper Limits for Iron Established by the IOM and Estimated Daily Intake of Iron from Food and Supplements Derived by the IOM (NHANES III, 1988-1994)

Life Stage Group		RDA for Iron (mg/person/day)[a]	UL for Iron (mg/person/day)[a]	Estimated Intake of Iron from Food and Supplements (mg/person/day)[b]	
				Mean	P90
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 18 years	27	45	48.97	88.84
	19 to 30 years				
	31 to 50 years				
Lactation	14 to 18 years	10	45	58.51	112.00
	19 to 30 years	9	45		
	31 to 50 years				

IOM = Institute of Medicine; P90 = 90th percentile; RDA = recommended daily allowance; UL = upper tolerable limit

[a] Obtained from IOM (2006)

[b] Obtained from IOM (2001).

Table 3.3-2 Recommended Daily Allowances and Tolerable Upper Limits for Phosphorous Established by the IOM and Estimated Daily Intake of Phosphorous Derived by the IOM (CSFII, 1994)

Life Stage Group		RDA for Phosphorous (mg/person/day)[a]	UL for Phosphorous (mg/person/day)[a]	Estimated Intake of Phosphorous (mg/person/day)[b]	
				Mean	P90
Children	1 to 3 years	460	3,000	943.9	1,280
	4 to 8 years	500	3,000	1,088	1,455
Males	9 to 13 years	1,250	4,000	1,407	1,993
	14 to 18 years			1,642	2,290
	19 to 30 years	700	4,000	1,659	2,258
	31 to 50 years		4,000	1,530	2,094
	51 to 70 years		4,000	1,307	1,789
	>70 years		3,000	1,191	1,587

Table 3.3-2 Recommended Daily Allowances and Tolerable Upper Limits for Phosphorous Established by the IOM and Estimated Daily Intake of Phosphorous Derived by the IOM (CSFII, 1994)

Life Stage Group		RDA for Phosphorous (mg/person/day)[a]	UL for Phosphorous (mg/person/day)[a]	Estimated Intake of Phosphorous (mg/person/day)[b]	
				Mean	P90
Females	9 to 13 years	1,250	4,000	1,203	1,579
	14 to 18 years			1,128	1,573
	19 to 30 years	700	4,000	1,031	1,429
	31 to 50 years		4,000	1,014	1,382
	51 to 70 years		4,000	986.5	1,325
	>70 years		3,000	874.3	1,181
Pregnancy	14 to 18 years	1,250	3,500	1,572	1,996
	19 to 30 years	700	3,500		
	31 to 50 years				
Lactation	14 to 18 years	1,250	4,000	1,496	1,741
	19 to 30 years	700	4,000		
	31 to 50 years				

IOM = Institute of Medicine; P90 = 90th percentile; RDA = recommended daily allowance; UL = upper tolerable limit

[a] Obtained from IOM (2006).

[b] Obtained from IOM (1997).

3.4 Estimated Intake of Iron, Phosphorus, and Casein from Food Uses of Iron Milk Proteinate

Iron milk proteinate is intended for use as a dietary source of iron in conventional food and beverage products in the U.S., and its uses will be fully substitutional to other iron ingredients currently on the U.S. marketplace. Iron milk proteinate will be used in the same food categories as other iron salts at levels based on cGMP in accordance with the principles of the U.S. FDA's fortification guidelines and Fortification Policy under 21 CFR §104.20 (U.S. FDA, 2019). Therefore, the intended conditions of use of iron milk proteinate will not change the current intakes of iron in the U.S. population.

As a worst-case, it was assumed that iron intakes from iron milk proteinate will be similar to iron intakes from food and supplements estimated by the IOM. In other words, a conservative approach was taken by assuming that all iron in the diet (natural and supplemental) is obtained from iron milk proteinate. This approach has been used previously for estimating dietary intake of iron from GRAS uses of sodium ferrous citrate described in GRN 441. Corresponding iron milk proteinate intakes were calculated based on estimated daily intakes of iron from food and supplements derived by the IOM (*i.e.*, assuming all iron intake from food and supplements is a result of iron milk proteinate consumption) and the average iron content of iron milk proteinate from proximate analyses (2.7%, see Table 2.4.1-1). As iron milk proteinate is an iron-casein-phosphate complex, corresponding phosphorous and casein intakes following 100% replacement of iron in the diet with iron from iron milk proteinate was also calculated based on the average content of phosphorus and protein from proximate analyses of iron milk proteinate (61% protein and 3.5% phosphorous, see Table 2.4.1-1). The estimated daily intakes of iron, iron milk proteinate, phosphorous and casein in the U.S. population from the intended conditions of use of iron milk proteinate using this conservative approach are presented in Table 3.4-1. In reality, the actual intakes of iron milk proteinate and iron, phosphorous, and casein from iron milk proteinate are expected to be much lower.

Among all individuals (including pregnant and lactating women), mean and 90th percentile intakes of iron from food and supplements, assuming 100% replacement of iron in the diet with iron from iron milk proteinate, is of 18.34 and 30.13 mg/person/day, respectively. Among individual population groups, mean iron intakes are highest in males 31 to 50 years of age at 21.09 mg/person/day, whereas 90th percentile iron intakes are highest in males >70 years of age at 34.50 mg/person/day. As indicated in Section 3.1, iron intakes from food and supplements at the mean and 90th percentile are below the UL established for iron by the IOM in all population groups expect pregnant and lactating women (see Table 3.3-1).

Corresponding iron milk proteinate intakes among all individuals (including pregnant and lactating women) at the mean and 90th percentile following 100% replacement of iron in the diet with iron from iron milk proteinate were calculated at 679 and 1,116 mg/person/day, respectively. Highest mean intakes were calculated at 781 mg/person/day in males 31 to 50 years of age and 1,278 mg/person/day in males >70 years of age.

Phosphorous intakes from the intended conditions of use of iron milk proteinate in this replacement scenario were calculated at 23.77 and 39.06 mg/person/day at the mean and 90th percentile among all individuals (including pregnant and lactating women), respectively. Highest mean intakes were calculated at 27.34 mg/person/day in males 31 to 50 years of age and 44.72 mg/person/day in males >70 years of age. In all population group, the estimated daily intake of phosphorous from iron milk proteinate at the mean and 90th percentile is well below the UL established for phosphorous by the IOM (see Table 3.3-2).

Casein intakes from the intended conditions of use of iron milk proteinate in this replacement scenario were calculated at 414 and 681 mg/person/day at the mean and 90th percentile among all individuals (including pregnant and lactating women), respectively. Highest mean intakes were calculated at 476 mg/person/day in males 31 to 50 years of age and 779 mg/person/day in males >70 years of age.

Table 3.4-1 Estimated Daily Intake of Iron from Food and Supplements and Corresponding Daily Intakes of Iron Milk Proteinate, and Phosphorous and Casein from Iron Milk Proteinate

Life Stage Group (maximum value/life stage group category)		Estimated Intake of Iron from Food and Supplements (mg/person/day)[a]		Estimated Intake of Iron Milk Proteinate (mg/person/day)[b]		Estimated Intake of Phosphorous from Iron Milk Proteinate (mg/person/day)[c]		Estimated Intake of Casein from Iron Milk Proteinate (mg/person/day)[d]	
		Mean	P90	Mean	P90	Mean	P90	Mean	P90
Children	1 to 3 years	10.36	17.60	384	652	13.43	22.81	234	398
	4 to 8 years	14.68	21.27	544	788	19.03	27.57	332	481
Males	9 to 13 years	18.05	25.70	669	952	23.40	33.31	408	581
	14 to 18 years	20.88	32.68	773	1,210	27.07	42.36	472	738
	19 to 30 years	20.87	31.84	773	1,179	27.05	41.27	472	719
	31 to 50 years	21.09	33.48	781	1,240	27.34	43.40	476	756
	51 to 70 years	20.64	34.30	764	1,270	26.76	44.46	466	775
	>70 years	20.95	34.50	776	1,278	27.16	44.72	473	779
Females	9 to 13 years	14.63	21.84	542	809	18.96	28.31	331	493
	14 to 18 years	13.24	19.61	490	726	17.16	25.42	299	443
	19 to 30 years	16.76	29.10	621	1,078	21.73	37.72	379	657
	31 to 50 years	17.11	31.01	634	1,149	22.18	40.20	387	701
	51 to 70 years	16.83	30.46	623	1,128	21.82	39.49	380	688
	>70 years	19.01	32.03	704	1,186	24.64	41.52	429	724

Table 3.4-1 Estimated Daily Intake of Iron from Food and Supplements and Corresponding Daily Intakes of Iron Milk Proteinate, and Phosphorous and Casein from Iron Milk Proteinate

Life Stage Group (maximum value/life stage group category)	Estimated Intake of Iron from Food and Supplements (mg/person/day)[a]		Estimated Intake of Iron Milk Proteinate (mg/person/day)[b]		Estimated Intake of Phosphorous from Iron Milk Proteinate (mg/person/day)[c]		Estimated Intake of Casein from Iron Milk Proteinate (mg/person/day)[d]	
	Mean	**P90**	**Mean**	**P90**	**Mean**	**P90**	**Mean**	**P90**
All individuals, including pregnant and lactating females	18.34	30.13	679	1,116	23.77	39.06	414	681

EDI = estimated daily intake; P90 = 90th percentile.

[a] Obtained from IOM (2001).

[b] Iron comprises 2.7% of iron milk proteinate on average (see proximate analyses in Table 2.4.1-1 of the dossier).

[c] Phosphorous comprises 3.5% of iron milk proteinate on average (see proximate analyses in Table 2.4.1-1 of the dossier).

[d] Iron milk proteinate is a complex of iron-casein-phosphate; protein comprises 61% of iron milk proteinate on average (see proximate analyses in Table 2.4.1-1 of the dossier).

PART 4. § 170.240 SELF-LIMITING LEVELS OF USE

No known self-limiting levels of use are associated with iron milk proteinate.

PART 5. §170.245 EXPERIENCE BASED ON COMMON USE IN FOOD BEFORE 1958

Not applicable.

PART 6. § 170.250 NARRATIVE AND SAFETY INFORMATION

6.1 Introduction

Iron milk proteinate is intended for use as a dietary source of iron in accordance with the Fortification Policy under 21 CFR 104.20 (U.S. FDA, 2019). The determination that iron milk proteinate is GRAS is on the basis of scientific procedures and the information supporting the safe use of iron milk proteinate include the following:

- Data pertaining to the identity, intended use, and estimated intake of iron milk proteinate;
- The expected metabolic fate of iron milk proteinate based on a published clinical study with iron milk proteinate (Henare *et al.,* 2019) and the available information on other inorganic iron sources (*e.g.,* ferrous sulfate); and
- Scientific and authoritative reviews and conclusions on the safety of the components of iron milk proteinate (*i.e.,* iron, phosphorus, and casein).

As discussed in Section 2.1, iron milk proteinate is a mineral-protein complex that consists of ferric iron bound to the phosphoserine residue of casein. The results of a published clinical study demonstrate iron milk proteinate to have a similar bioavailability as ferrous sulfate, and its absorption is regulated by iron stores. The results are further corroborated by *in vitro* studies that demonstrate that iron milk proteinate is soluble, is readily digested to peptides, and has similar iron bioaccessibility as ferrous sulfate. The available information supports that iron milk proteinate is absorbed from the diet into the enterocytes along the gastrointestinal tract and added to the intracellular iron pool, where it is then handled in a similar manner as other inorganic (non-heme) dietary sources of iron. The metabolic fate of non-heme iron is well characterized (*i.e.,* absorption is impacted by solubility and stabilization of iron in its divalent form). The systemic exposure from ingestion of iron milk proteinate will be limited to the component amino acids, iron, and phosphate. The safety of iron is generally recognized and has been evaluated by multiple scientific and authoritative bodies (INACG, 1993; EVM, 2002, 2003a; IOM, 2006) and discussed in previous GRAS notifications that received no objections from the FDA (see Section 3.1.1). Therefore, the safety of iron milk proteinate is supported by the metabolic fate and published clinical data demonstrating equivalence to other dietary forms of iron (*e.g.,* ferrous sulfate).

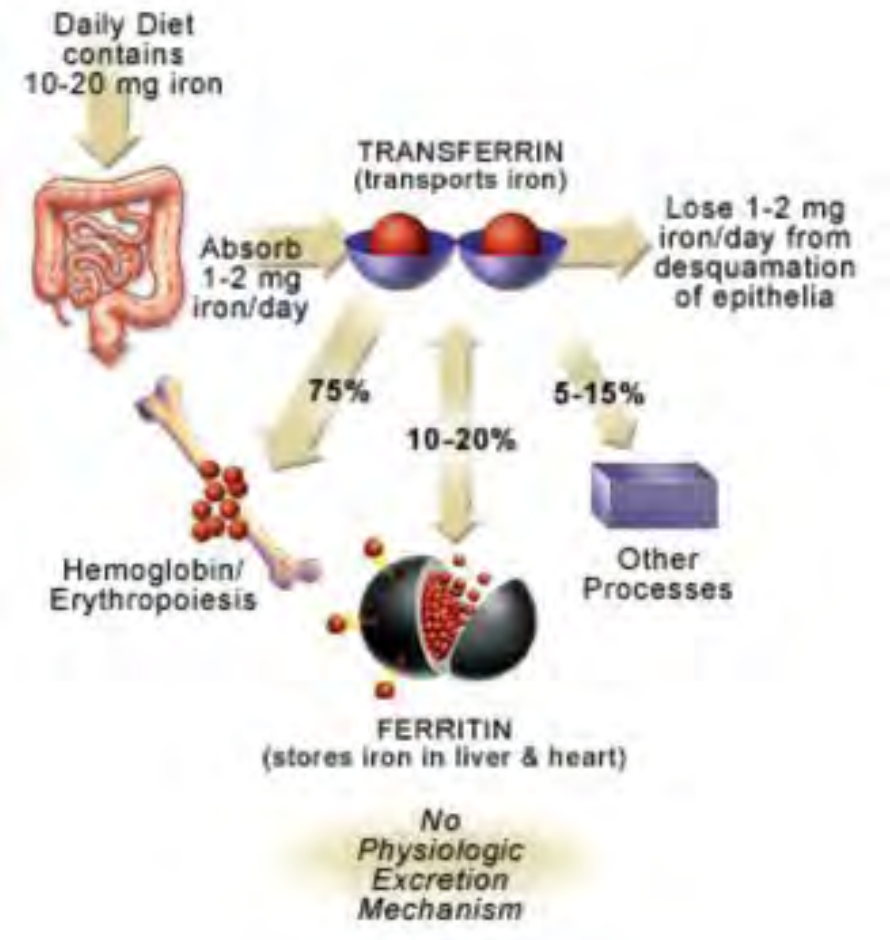
The IOM (2006) reviewed the safety of iron and established an UL of 45 mg/day for males and females over 14 years of age and 40 mg/day for individuals younger than 14 years based gastrointestinal effects reported in Swedish individuals consuming 70 mg/day of supplemental iron salts (Frykman *et al.,* 1994). A manual search of the PubMed database was conducted to identify published scientific literature available since the IOM review pertaining to safety-related endpoints in humans consuming ferrous sulfate. A systematic review and meta-analysis of ferrous sulfate supplementation and gastrointestinal effects was identified, in which the authors reported a significant increase in gastrointestinal effects that was not associated with dose (Tolkien *et al.,* 2015). The results of the updated literature search indicate that there have not been any published studies since the last IOM review that would contradict the previous safety conclusions, and therefore, use of the UL of 45 mg/day would be appropriate in the safety evaluation of iron from the proposed uses of iron milk proteinate.

The data was reviewed by a panel of experts, qualified by their scientific training and experience in the safety evaluation of food ingredients, who concluded that the intended uses of iron milk proteinate are safe and suitable and would be GRAS based on scientific procedures (see Appendix A).

6.2 Absorption, Distribution, Metabolism, and Excretion

Iron milk proteinate is a mineral-protein complex that consists of ferric iron (Fe^{3+}) linked to phosphate residues of caseins and stabilized by inorganic phosphate (see Figure 2.1-1). The results of *in vitro* trials with iron milk proteinate demonstrate that the majority of iron does not dissociate from the iron milk proteinate complex at pH 2.0 due to the coordination bonds between iron and phosphoserines, and therefore, remain bound to soluble caseins (see Section 6.2.1 for further details). Under gastric conditions (pH 1.7), iron from iron milk proteinate was demonstrated to be as soluble as ferrous sulfate (Henare *et al.,* 2019). The available data suggests that iron from iron milk proteinate remains bound and soluble and has a similar iron bioaccessibility as ferrous sulfate. Thus, the iron-peptide complex is soluble and facilitates to the apical membrane, where it is transported into the enterocyte. Once inside the enterocyte, the iron from iron milk proteinate is dissociated, added to the intracellular iron pool, and is expected to be processed similar to other iron compounds.

Figure 6.2-1 Iron Cycle in the Human Body (Taken from Abbaspour *et al.,* 2014)

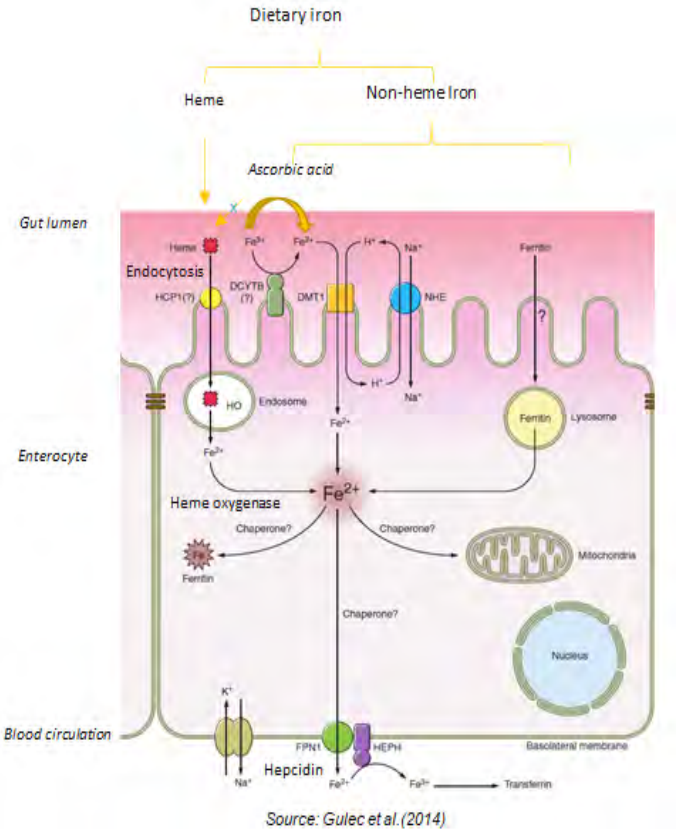


The metabolic fate of iron was discussed in detail in GRN 271 and 441 and is incorporated by reference and briefly discussed as follows (U.S. FDA, 2009, 2013). Iron is an essential element that is recycled and highly conserved by the human body (see Figure 6.2-1; Abbaspour *et al.,* 2014). The fraction of iron absorbed

from the human diet ranges from 5 to 35%, with approximately 1 to 2 mg of iron absorbed per day; menstruating women absorb up to 3.4 mg/day and pregnant women may absorb as high as 5 mg/day near the end of pregnancy (IOM, 2006). The absorption of iron from the diet is dependent on the existing iron stores in the body; less iron is absorbed from the diet in individuals with high iron stores (IOM, 2006).

There are two pathways for the absorption of iron in humans. One pathway is specific for the absorption of heme iron derived from hemoglobin and myoglobin in meat sources, and the other pathway allows for the absorption of nonheme iron, (*e.g*., iron salts) sources provided by plant and dairy foods (Table 6.2-2). Non-heme iron is the predominant form of dietary iron consumed by humans. Non-heme iron is transported into the enterocytes of the duodenum and jejunum *via* the divalent metal transporter 1 (DMT1) (IOM, 2006; Abbaspour *et al.,* 2014; Nishito and Kambe, 2018). Iron must be transported in its reduced state as ferrous iron (Fe^{2+}), and therefore dietary sources of ferric iron (Fe^{3+}) such as iron milk proteinate must be reduced by ferrireductase duodenal cytochrome B at the apical membrane to enable absorption *via* the DMT1 transporter. Ferric iron can also be reduced by dietary ascorbic acid, which forms a soluble reduced iron-chelate complex in the stomach (IOM, 2006). Iron absorption from meals can be increased by as much as three- to six-fold when 50 mg of ascorbic acid is consumed. Ferrous ions are stored as ferritin, and transfer across the basolateral membrane into the plasma is mediated by the membrane transporter ferroportin.

Figure 6.2-2 Mechanism of Iron Absorption (Gulec *et al*., 2014)



Source: Gulec et al.(2014)

Iron is used in the synthesis of oxygen transport proteins, such as hemoglobin and myoglobin, or in the regulation of cellular metabolism of iron. Excess iron is stored as ferritin in the liver, spleen, and bone marrow. There is no known physiological excretion mechanism for iron, and therefore, absorption is strictly regulated *via* negative feedback of the peptide hormone, hepcidin. This protein controls ferroportin expression on the basolateral membrane; high concentrations of hepcidin increase ferroportin

internalization, thus reducing plasma levels of iron. As discussed in GRN 441, the excretion of iron is dependent on the homeostatic degradation process of erythrocytes, wherein iron is recycled into new erythrocytes. Thus, in the absence of bleeding or pregnancy, only a small amount of iron is loss on a daily basis (up to 1 mg/day in non-menstruating women) (IOM, 2006). Iron is primarily excreted through the feces *via* cell desquamation, and lesser amounts in the urine and bile. The risk of systemic iron overload from dietary sources is negligible in individuals with normal intestinal function (EFSA, 2015).

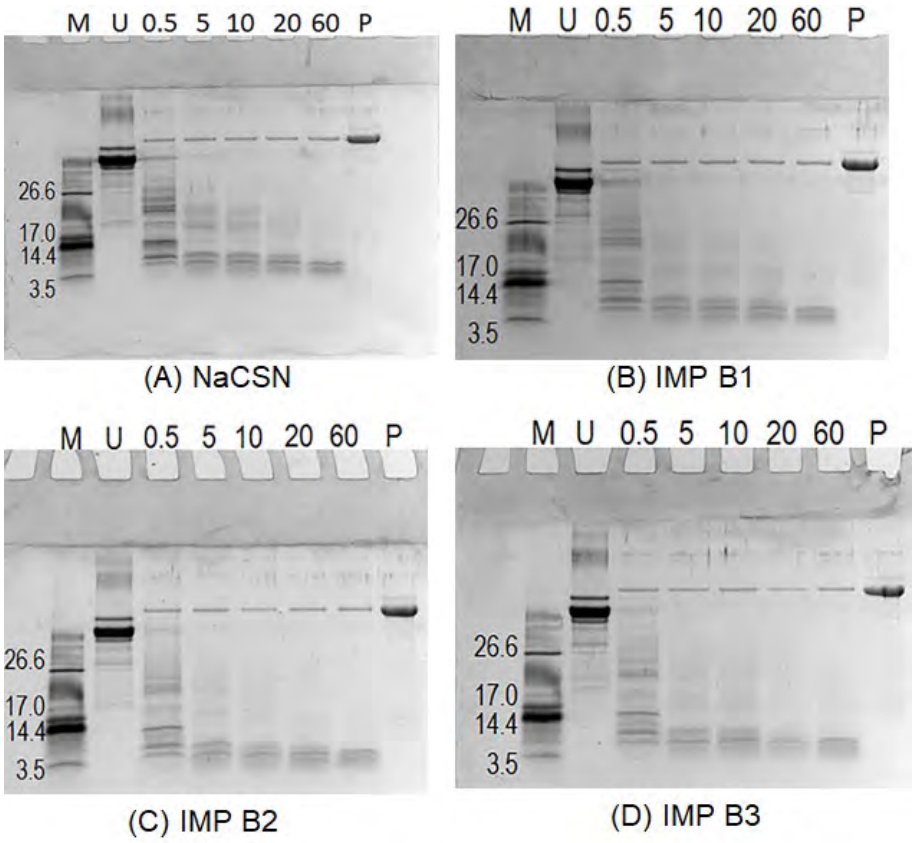
6.2.1 *In vitro* Studies of Iron Milk Proteinate

6.2.1.1 Digestibility of Iron Milk Proteinate

The digestibility of 3 batches of iron milk proteinate (Lot No. 742344, 742376, 742382) was investigated in an *in vitro* model mimicking digestion under gastric conditions. The study was conducted using the protocol described by Minekus *et al.* (2014). Briefly, aqueous solutions of iron milk proteinate (1% w/v) were mixed with SGF at pH 2.0 and pre-incubated at 37°C for 15 minutes. Next, digestion was initiated by the addition of a stock solution of 10 mg/mL pepsin containing 3,500 U/mg to the iron milk proteinate mixture to achieve a final activity of 2,000 U pepsin/mL. Digested samples were collected at 0, 0.5, 5, 10, 20, and 60 minutes. The digested samples were then diluted in reducing tricine sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer (2% w/v SDS, 200 mM DTT, pH 8.45) to obtain a final protein concentration of approximately 1 mg/mL. The samples were analyzed by SDS-PAGE according to the protocol described by Dave *et al.* (2013). The full study report is provided in Appendix B.

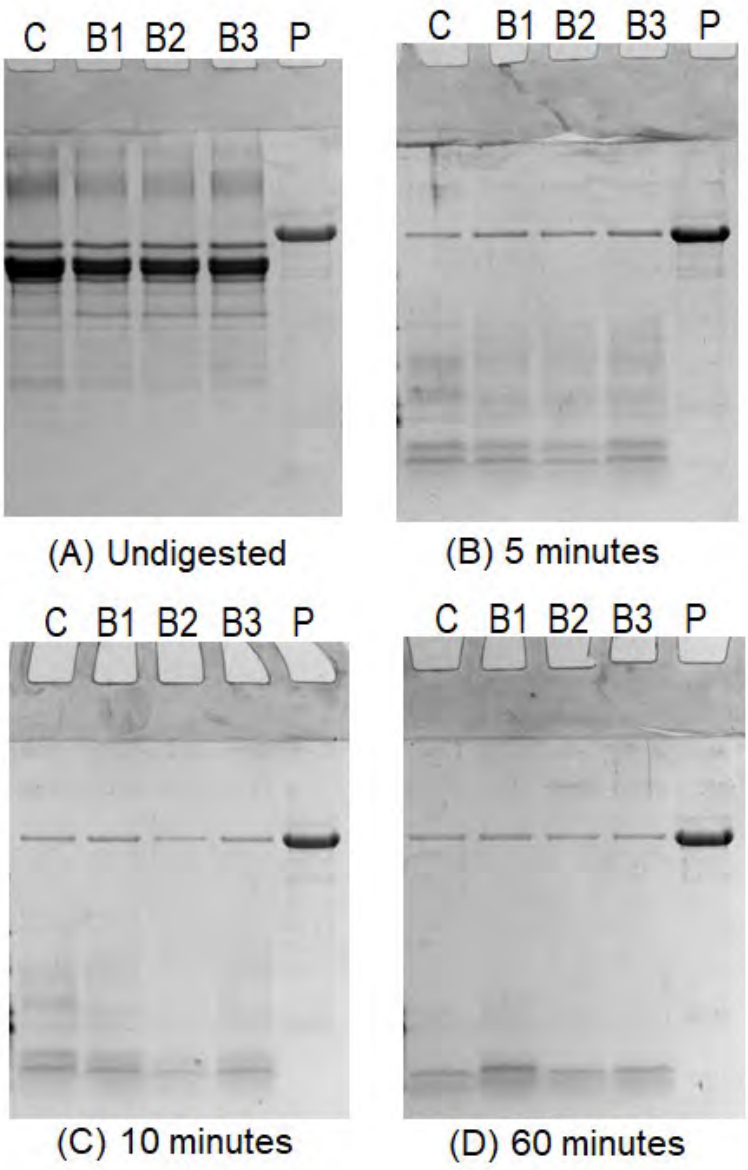
As shown in Figures 6.2.1.1-1 and 6.2.1.1-2, iron milk proteinate was digested in a similar manner as sodium caseinate. The samples were completely hydrolyzed by 5 minutes and only small peptides were detected after 60 minutes of digestion. Some polypeptide bands eluted at molecular weights lower than κ-casein, which were attributed to residual whey proteins in the caseinate sample. All caseinate samples demonstrated significant hydrolysis within 30 seconds of incubation and unhydrolyzed casein fractions were not detected after 5 minutes of digestion. The results of this study indicate that the caseinate component of the iron milk proteinate complex will be hydrolyzed into small peptides. The rate of hydrolysis of casein fractions and their hydrolysis pattern in iron milk proteinate were comparable to that of sodium caseinate. Thus, it was concluded that iron milk proteinate would be digested under gastric conditions in a similar manner as sodium caseinate to yield peptides and amino acids.

Figure 6.2.1.1-1 SDS-PAGE Results of (A) Sodium Caseinate and (B, C, D) 3 Batches of Iron Milk Proteinate Under Stimulated Gastric Conditions (pH 2, 37°C)



IMP B1, B2, B3 = batches 1 to 3 of IMP; NaCSN = sodium caseinate; P = pepsin digested; U = undigested.

Figure 6.2.1.1-2 Hydrolysis Patterns of (A) Sodium Caseinate and (B, C, D) 3 Batches of Iron Milk Proteinate Under Stimulated Gastric Conditions (pH 2, 37°C) at 5, 10, and 60 Minutes



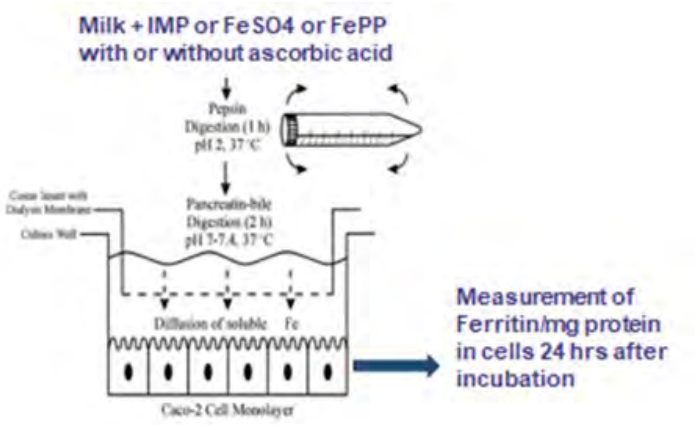
B1, B2, B3 = batches 1 to 3 of IMP; NaCSN = sodium caseinate; P = pepsin digested; U = undigested.

6.2.1.2 Bioaccessibility of Iron from Iron Milk Proteinate

The bioaccessibility of iron from iron milk proteinate when added to milk was investigated in an *in vitro* model of simulated digestion coupled with Caco-2 cells (Sabatier *et al.,* 2020). In this study, iron milk proteinate, ferrous sulfate, or ferric pyrophosphate was added to milk with and without ascorbic acid. The mixture was subject to digestion with pepsin for 1 hour at pH 2.0 and 37°C and pancreatin-bile digestion for 2 hours at pH 7.0 to 7.4 and 37°C. The digest was placed in a dialysis chamber limiting diffusion to small molecular weight compounds (*e.g.*, soluble iron) across the dialysis membrane for uptake by Caco-2 cells.

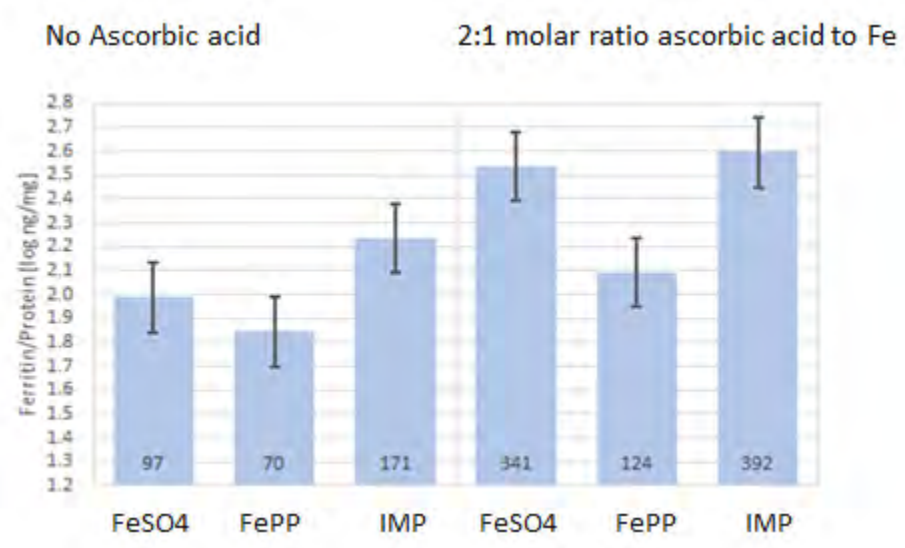
Ferritin formation by Caco-2 cells were measured as a marker for cellular iron uptake and an indicator of iron bioavailability. The study was performed using similar methodology as Glahn *et al.* (1998) and Yun *et al.* (2004) (Figure 6.2.1.2-1).

Figure 6.2.1.2-1 Diagram of *In Vitro* Digestion and Bioaccessibility in Caco-2 Cells



A trend towards higher bioaccessibility of iron was observed in the iron milk proteinate samples compared to ferrous sulfate and ferric pyrophosphate samples (see Figure 6.2.1.2-2); however, this effect was abrogated in the presence of ascorbic acid, demonstrating that iron milk proteinate facilitates the transfer of iron complexes in soluble form to the apical membrane of the Caco-2 cell monolayer. The results of this study indicated that part of iron from iron milk proteinate is released (*ca.* 10%), while a portion remained bound to the phosphoserine fraction (*ca.* 90%). Considering that the iron uptake by the Caco-2 cells was not significantly different in the presence of ascorbic acid demonstrates that the maximum bioaccessibility potential of iron from iron milk proteinate is comparable to iron sulphate.

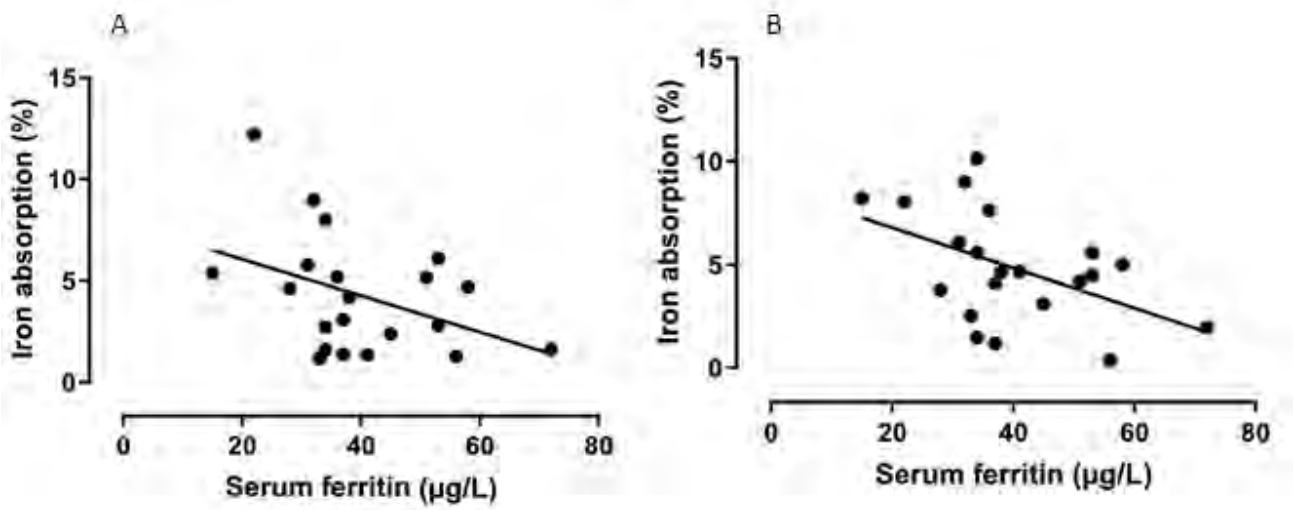
Figure 6.2.1.2-2 Effect of Ascorbic Acid on the Bioaccessibility of Iron from Iron Milk Proteinate (IMP), Ferrous Sulfate ($FeSO_4$), and Ferric Pyrophosphate (FePP)



6.2.2 Bioavailability of Iron from Iron Milk Proteinate

The bioavailability of iron from an iron-casein complex (*i.e.,* iron milk proteinate) was investigated in a randomized, comparator-controlled trial with a crossover design (Henare *et al.*, 2019). Twenty-one healthy women (aged 25.2±5.7 years) with normal iron status were provided with pasteurized whole milk containing 2.5 mg of isotopically labelled iron as iron-casein complex (^{57}Fe) or ferrous sulfate (^{58}Fe). Blood samples were collected at baseline and 14 days after the consumption of each drink containing the labelled iron for analysis of erythrocyte incorporation of the latter and calculation of the fractional iron absorption. The fractional absorption of iron, either as ^{57}Fe or ^{58}Fe from iron-casein complex or ferrous sulfate, respectively, was not statistically significant between the treatment groups. A significant linear relationship between fractional iron absorption and serum ferritin concentration was observed (Figure 6.2.2-1). The inverse relationship between iron absorption and serum ferritin has been reported for other dietary sources of iron and is consistent with the negative feedback regulation of iron absorption and transport based on iron status (Hurrell *et al.,* 2010). The rate of iron absorption from iron milk proteinate and ferrous sulfate was 3.5% and 3.9%, respectively, translating to a relative iron bioavailability of approximately 87%. The slopes of the linear regressions were not statistically different between the 2 sources of iron, suggesting that iron absorption from iron milk proteinate is regulated by iron stores similar to iron from ferrous sulfate. Therefore, it is expected that the relative iron bioavailability from iron milk proteinate and ferrous sulfate will be similar and consistent across individuals of different iron status.

Figure 6.2.2-1 Iron Absorption from Iron Milk Proteinate (A) and Ferrous Sulfate (B) in Healthy Women with Normal Iron Status



6.2.3 Metabolic Fate of Phosphate from Iron Milk Proteinate

Iron milk proteinate is composed of both inorganic and organic forms of phosphate, similar to food phosphorus. As previously mentioned, complete hydrolysis of iron milk proteinate is observed under gastric conditions and inorganic phosphate is assumed to dissociate in the gastrointestinal lumen (EFSA, 2019).

Absorption of phosphorus mainly occurs in the form of inorganic phosphate as free orthophosphate in the intestinal tract, as organic phosphates are hydrolyzed by intestinal phosphatases (IOM, 1997; EFSA, 2019). The majority of phosphorus absorption occurs by passive, concentration-dependent processes, with a small amount of phosphorus absorption occurring by saturable, active transport (IOM, 1997; EFSA, 2019). Approximately 55 to 70% of dietary phosphorus is absorbed in the small intestine of adults and approximately 65 to 90% in infants and children, while dietary intake does not affect absorption efficiency (IOM, 1997, 2006; EFSA, 2019). In addition, no apparent adaptive mechanisms are known to improve absorption of phosphorous with low dietary intakes (IOM, 1997). Once absorbed, phosphorus and calcium are distributed together in the skeleton (*i.e.,* hydroxyapatite), which makes up approximately 85% of the skeletal content and 15% in soft tissue (IOM, 2006; EFSA, 2019). Phosphorus is mainly excreted through the kidneys, with lesser amounts excreted in shed cells of skin and intestinal mucosa (IOM, 1997; EFSA, 2019). The amount of phosphorus excreted in the urine is equivalent to the amount absorbed through the diet (IOM, 2006).

6.2.4 Metabolic Fate of Casein/Caseinate from Iron Milk Proteinate

Casein and caseinates are consumed in the human diet primarily as milk proteins, which comprise up to 3.5% of cow's milk (Jahan-Mihan *et al*., 2011). Caseins exist as αs1-, αs2-, β-, and κ-caseins and are referred to as casein micelles (*e.g.,* large colloidal aggregates). Casein and caseinates are hydrolyzed by proteinases, such as pepsin, trypsin, and chymotrypsin, yielding smaller peptides, which can be further hydrolyzed by pancreatic peptidases to yield free, di-, tri-, or oligo-amino acids that are then absorbed.

6.2.5 Safety of the Individual Components of Iron Milk Proteinate

The safety of iron milk proteinate is supported by the fact that its components, iron, phosphate, and casein, are GRAS substances with a long history of safe consumption in the human diet. As discussed, iron milk proteinate is intended for use as a dietary source of iron. The information presented in Section 6.2 demonstrate that iron from iron milk proteinate has a similar bioaccessibility potential and comparable bioavailability to other permitted forms of inorganic iron used in the U.S. diet, such as ferrous sulfate. Iron from iron milk proteinate will be absorbed in a similar manner as all dietary forms of non-heme iron (*i.e.,* through the DMT1 transporter) by the enterocytes of the duodenum and jejunum. Iron from iron milk proteinate absorbed into the enterocyte is bound to ferritin and contributes to the intracellular pool of iron where it is subject to normal physiological processes regulating iron status. The acute toxicity of iron milk proteinate was investigated in Wistar rats (Section 6.3.1) and there were findings in these studies to suggest that the acute toxicity of iron from iron milk proteinate is greater than other iron salts.

As the proposed uses of iron milk proteinate is intended to be fully substitutional to other iron fortificants currently on the U.S. marketplace at levels in accordance with cGMP and the Fortification Policy (21 CFR §104.20), its conditions of use is not expected to significantly affect the current intakes of its components, iron, phosphorus, and casein, in the U.S. population (U.S. FDA, 2019). The dietary exposures to the components of iron milk proteinate were estimated and discussed in Section 3.0. The estimated daily intake of iron from the proposed food uses of iron milk proteinate was up to 34.5 mg/day, while the highest estimated daily intake of phosphorus was 44.5 mg/day. The proposed food uses of iron milk proteinate do

not appreciably increase the U.S. population's existing exposure to phosphorus and is therefore not expected to pose any safety concerns (IOM, 1997). The dietary exposures to casein/caseinate from the proposed food uses of iron milk proteinate is not expected to pose any safety concerns given that these compounds are digested into small peptides and individual amino acids, similar to other dietary proteins. Thus, the safety evaluation of iron milk proteinate is focused on the safety of iron. As the estimated daily intake of iron was below the UL of 45 mg/day for males and females over 14 years of age and 40 mg/day for individuals younger than 14 years, the proposed food uses of iron milk proteinate as a substitute for other iron fortificants will not pose any safety concerns (IOM, 2006). The USDA noted that a small proportion of users of iron supplements have intakes that are above the UL; however, the adverse effects are not well defined. Furthermore, iron supplementation was noted to be very common in early childhood and pregnancy and is unlikely to pose a health risk (USDA, 2015).

6.2.5.1 Iron

The safety of iron has been the subject of multiple comprehensive evaluations by various scientific and authoritative bodies (JECFA, 1983; INACG, 1993; EVM, 2002, 2003a; IOM, 2006). JECFA established a provisional maximum tolerable daily intake (PMTDI) of 0.8 mg/kg body weight/day for iron from all sources except iron oxide coloring agents, supplemental iron for pregnancy and lactation, and supplemental iron for specific clinical requirements, based on a generally available evidence that supplemental iron intake of 50 mg/day has been reported to be safe and well tolerated by healthy individuals for long periods of time (JECFA, 1983). JECFA estimated the average daily intake of iron to be in the range of 17 mg/day for males aged 20 to 34 and 9 to 12 mg/day for all females. The European Food Safety Authority (EFSA) reviewed information on iron in order to establish dietary reference intakes (population reference intakes, average requirements, adequate intakes, and reference intake ranges) and maintained their conclusion that no UL could be established for iron (EFSA, 2015). EFSA noted that the adverse gastrointestinal effects reported after short-term ingestion of non-heme iron at doses of 50 to 60 mg/day are not suitable to establish a UL for iron from all sources. EFSA also concluded that there was inadequate data to establish a UL for iron based on systemic overload due to inadequate data to enable the construction of response curves between intake, body burden, homeostatic adaptations, and adverse health effects. EFSA also concluded that were was inadequate data to demonstrate causal relationships between excess iron intake and increased risk of various chronic diseases such as cardiovascular diseases, diabetes and cancer.

Based on a review of the safety of iron, the IOM concluded that excessive intake of iron from the diet is low in the general population; however, the following adverse effects have been associated with consumption of high levels of iron (IOM, 2006):

- Acute toxicity characterized by vomiting and diarrhea, followed by cardiovascular, central nervous system, kidney, liver, and hematological effects;
- Reduced absorption of other minerals, such as zinc;
- Gastrointestinal effects such as constipation, nausea, vomiting, and diarrhea;
- Secondary iron overload resulting from increased body iron stores or hematological disorders that increase the rate of iron absorption;
- Potential risk factor in coronary heart disease; and
- Increased risk for hepatocellular carcinoma in individuals with hereditary hemochromatosis and cirrhosis.

The IOM noted that acute adverse effects occur at doses between 20 and 60 mg/kg, however, acute intake data are not considered in setting an UL (IOM, 2006). Likewise, as there was no evidence of clinically significant adverse effect associated with iron-zinc interactions, the data was not considered in setting the UL. The totality of evidence for the risk of coronary heart disease and cancer and dietary iron intake was inconclusive, and therefore, the IOM selected gastrointestinal side effects as the critical adverse effect to base the UL for iron (IOM, 2006). It was noted that gastrointestinal effects were generally observed in individuals consuming high levels of supplemental iron on an empty stomach, rather than in individuals consuming high levels of iron in the diet. As discussed in GRN 441, "*the risk of iron overload and any associated adverse effects as a result of the consumption of foods fortified with iron is very low."* (U.S. FDA, 2013). In setting the UL, the IOM used a culmination of the lowest-observed-adverse-effect level (LOAEL) of 70 mg/day from supplemental iron salts in Swedish individuals (*i.e.,* 60 mg/day) (Frykman *et al.,* 1994) and the intake of iron from the diet (11 mg/day) in the European population (IOM, 2006). A tolerable UL of 45 mg/day was set for males and females over 14 years of age and 40 mg/day for individuals younger than 14 years.

A manual review of the published scientific literature was conducted through May 2020 using the PubMed database to identify studies published since the review by IOM in 2006 evaluating safety-related endpoints in humans consuming ferrous sulfate. The search terms included "ferrous sulfate" and "adverse effect*" and "tolerability" and identified 21 studies conducted with adults (see Appendix D for search summary). A systematic review and meta-analysis of ferrous sulfate supplementation and gastrointestinal effects indicated that ferrous sulfate consumption was associated with a significant increase in gastrointestinal side effects, however, the finding was not associated with dose (Tolkien *et al.,* 2015). It was noted that the increase in odds ratio was greater than 1.0 at doses greater than 120 mg/day of elemental iron. The results of these studies are supportive of the existing safety conclusions of iron by the IOM and therefore, the safety evaluation of iron from the proposed food uses of iron milk proteinate was conducted using the UL of 45 mg/day.

As previously discussed, the intended uses of iron milk proteinate will be substitutional to existing iron fortificants and will not increase the current dietary exposures to iron. As shown in Table 3.4-1, the highest estimated intake of iron from food and supplements was 34.5 in males over 70 years of age; therefore the dietary intakes of iron in the U.S. population arising from current iron fortification practices in the United States is below the UL of 45 mg/day as established by the IOM. Since food uses of iron milk proteinate will be substitutional to current dietary sources of iron added to the diet, uses in accordance with FDA's food fortification policy will not result in excessive intake of iron that would be of safety concern.

6.2.5.2 Phosphate

The safety of phosphates has been extensively reviewed by a number of authoritative bodies, including the European Commission's Scientific Committee on Food (SCF, 1978, 1991, 1994, 1997) and by JECFA (1974, 1982, 2002). In 1982, JECFA derived a maximum tolerable daily intake (MTDI) of 70 mg/kg body weight as phosphorous for phosphorous and polyphosphates from all sources based on the fact that phosphorous is an "essential nutrient and unavoidable constituent of food" (JECFA, 1982). SCF concurred with JECFA's conclusions and allocated an acceptable daily intake (ADI) of "not specified". EVM (2003b) concluded that an intake of 2,400 mg/day, equivalent to 2,110 mg/day as inorganic phosphorous, from food, including food additives and water, and 250 mg/day from supplements, was without adverse effects. More recently, the safety of phosphates, including phosphoric acid, di-, tri-, and polyphosphates, was reviewed by the European Food Safety Authority (EFSA) Panel on Food Additives and Flavourings added to Food (FAF Panel) in 2019 (EFSA, 2019). EFSA considered the group of phosphates to be of limited toxicity owing to the fact that it is an essential nutrient for all living organisms. In a series of short-term, subchronic,

and chronic toxicity studies conducted with various phosphates and polyphosphates, the only reported significant adverse effect of excessive phosphate consumption is calcification of the kidney and tubular nephropathy. In human clinical studies, impairment of renal function was reported following consumption of doses up to 4,800 mg/day, equivalent to 68.6 mg/kg body weight/day for a 70-kg individual, while no such kidney effects were reported following daily consumption of 2,000 mg phosphorous, equivalent to 28.6 mg/kg body weight/day. The FAF Panel noted that the group of phosphates and polyphosphates is not of genotoxic or carcinogenic concern and do not pose any risk of reproductive or developmental toxicity.

The FAF Panel derived a no-observed-adverse-effect level (NOAEL) of 76 mg/kg body weight/day based on the results of a chronic rat study with sodium triphosphate (Hodge, 1960 [unpublished]), and combined with the background dietary intake of phosphorous of 91 mg/kg body weight/day, reported a NOAEL of 167 mg/kg body weight/day as phosphorous. Using the NOAEL of 167 mg/kg body weight/day, the FAF Panel determined an ADI of 40 mg/kg body weight/day for phosphorous. The ADI is equivalent to a daily exposure of 2,800 mg/day for a 70-kg individual, which is within the UL for phosphorous of 4,000 mg/day (IOM, 2001).

6.2.5.3 Casein

Casein and caseinates are natural components of milk and have an extensive history of safe consumption in the human diet. The safety of casein and caseinates (calcium, sodium) was reviewed by SCOGS, who concluded the following:

- "*It is essential that food grade specifications for casein, sodium caseinate, and calcium caseinate be established including provisions for acceptable levels of lysinoalanine, nitrite, and nitrosamines. Assuming that acceptable levels of lysinoalanine, nitrite, and nitrosamine are established, there is no evidence in the available information on casein, sodium caseinate, or calcium caseinate that demonstrates or suggest reasonable grounds to suspect a hazard when they are used at levels that are now current or that may reasonably be expected in the future*".

- "*There is no evidence in the available information on casein that demonstrates or suggests reasonable grounds to suspect a hazard when it is used in paper and paperboard products for food packaging at levels that are now current or that might reasonably be expected in the future*".

6.2.6 Other Information Applicable to the Safety Evaluation

6.2.6.1 Acute Toxicity of Iron Milk Proteinate

An acute oral toxicity study was conducted in Wistar rats to investigate the acute toxicity of iron milk proteinate (FerriPro 2). This study was conducted in accordance with Organisation for Economic Co-operation and Development (OECD) Test Guideline No. 423 (OECD, 2001). The full study report is provided in Appendix C. Iron milk proteinate (Lot No. 19FP204[4]) was mixed with water and administered by gavage to fasted female Wistar rats (n=6; age 11 to 12 weeks; body weight 198 to 218.7 g). A single gavage dose of the test item was administered orally to a group of experimental animals at one of the defined doses (*i.e.,* 300 mg/kg body weight) as a first step (G1-FTS). As all the rats survived at this step, the test was continued at the same dose of 300 mg/kg bodyweight (G1-STS), all the rats survived at this step, the test was continued at the next higher dose of 2,000 mg/kg bodyweight (G2-FTS), all the rats survived at this step, hence the test was confirmed with three additional animals with the same dose of 2,000 mg/kg body weight (G2-STS). No test item-related mortality was observed, and hence testing was stopped and the LD_{50} cut-off

[4] This batch of iron milk proteinate was comprised of 65% protein, 3.8% phosphorus, and 2.8% iron.

value was determined. Animals were observed for clinical signs and mortality for 14 days. Body weights were measured prior to dosing on Day 1 and on Days 8 and 15. Necropsy was performed in all animals at the end of the study period. No adverse clinical signs or animal mortality were observed. In addition, no gross pathological changes were observed in any animal. Based on the results of this study, it was concluded that iron milk proteinate has an LD_{50} of >2,000 mg/kg. The iron content of iron milk proteinate is *ca*. 3%, therefore the administered dose of iron in the study was 60 mg/kg body weight. Although the study was not intended to evaluate the LD50 of iron from iron milk proteinate, other reported LD50 values for various iron salts in rats have been reported to range from 28 mg iron/kg body weight (ferric chloride) to 2329 mg iron/kg body weight for ferrous fumarate (JECFA, 1983). The findings in the acute toxicity study therefore do not provide evidence that provision of iron as iron milk proteinate is more acutely toxic than other iron salts that have been tested.

6.2.6.2 Animal Safety Studies on Ferrous Sulfate

The toxicity of iron is largely dependent on the amount of iron absorbed. The information discussed in Section 6.2 demonstrated that iron milk proteinate shares a similar absorption and bioavailability to ferrous sulfate, and therefore, animal toxicity studies of ferrous sulfate are relevant to the safety evaluation of iron milk proteinate. This rationale was previously used in the safety evaluation of ferrous ammonium phosphate, an inorganic source of iron, as discussed in GRN 271. A number of studies evaluating the acute toxicity, subchronic toxicity, and developmental toxicity and teratogenicity studies on ferrous sulfate was discussed in GRN 271 and GRN 441 and are incorporated by reference. The key findings from these studies are summarized in Table 6.3.3-1. Ferrous sulfate was reported to be of low acute oral toxicity, with LD_{50} values of 670, 1,720, 1,028, and over 1,000 mg/kg body weight, equivalent to 134, 344, 206, and over 200 mg iron/kg body weight, in mice, rats, rabbits, and dogs, respectively (Keith, 1957; Boccio *et al.,* 1998). The mortality rates of Wistar rats (20 to 40/sex/group) administered a single dose of 750 mg iron/kg body weight by gavage ranged from 40 to 95% (Berkovitch *et al.,* 1997).

In the repeated-dose toxicity studies, the most commonly reported adverse effects were hepatic-related, specifically iron content and deposition and indicators of liver toxicity (*e.g.,* ALT, ALP) (Omara *et al.,* 1993; Omara and Blakley, 1993; Appel *et al.,* 2001). However, no accompanying histopathological findings were reported in the liver or spleen. The NOAEL for ferrous sulfate was determined to be in the range of 11.54 mg/kg body weight/day as iron in a feeding study in Sprague-Dawley rats (Appel *et al.,* 2001) and 450 mg/kg body weight/day as iron in a feeding study with male weanling CD-1 mice (Omara *et al.,* 1993; Omara and Blakley, 1993). No significant adverse findings on developmental toxicity parameters were reported at doses of 110 mg iron/kg body weight/day in Wistar rats (Fairweather-Tait *et al.,* 1984) and no maternal toxicity or teratogenicity findings were reported in mice and rats at doses up to 160 and 200 mg/kg body weight/day, respectively (FDRLI, 1974).

Assuming a typical serving of food to which iron milk proteinate may be added will contain approximately 6 mg of ferric iron, the maximum dietary intake of iron by a heavy consumer ingesting 3 servings of iron milk proteinate per day would be 18 mg of iron, which corresponds to an intake of 0.3 mg/kg on a body weight basis. Nestle notes that the dietary intake of iron from typical food uses of IM iron milk proteinate P result in dietary exposures on a body weight basis that are below NOAEL values that have been derived for ferrous sulphate, a highly bioavailable source of iron. Based on the availability of a suitable clinical data set that was used to derive a tolerable upper intake level for iron, derivation of a margin of safety for iron intake relative to NOAEL values from animal toxicity studies was not considered necessary or useful for the safety assessment of iron milk proteinate.

Table 6.3.3-1 Key Findings of Toxicity Studies on Ferrous Sulfate (Adapted from GRN 271)[a]

Test Species	Dose/Concentration	Duration	Results[b]	Reference
Subchronic Toxicity				
Mice (BABL/c) 5 F[c]/group	3.75 or 37.5 mg iron/kg bw/day	1 to 2 weeks	• NSD in body weight, hematocrit, or hemoglobin concentration • ↑ hepatic iron level in infant but not adult animals • ↑ frequency of occurrence of secondary lysosomes in intestinal epithelial cells, intercellular junction opening between cells, and eosinophilic leukocytes outside the basement membrane that were considered to be a result of dietary iron overload	Hirohata *et al.* (1998)
Mice (CD-1) 5 to 8 M/group	18, 750, or 1,200 mg iron/kg bw/day	7 weeks	• NSD in hematocrit, water consumption, relative spleen, heart, or kidney weights • ↓ body weight (1,200) • ↑ liver weight (1,200) • ↑ iron content of hepatocytes, Kupffer cells, and splenic macrophages, and ALT and ALP (750, 1,200) • No gross lesions in liver, spleen, heart, kidney, or pancreas in macroscopic examination	Omara *et al.* (1993)
Mice (CD-1) 5 to 8 M/group	18, 450, 750, or 1,200 mg iron/kg bw/day	7 weeks	• ↑ relative liver weight (750, 1,200) • ↓ body weight (1,200) • ↑ ALT activity (1,200) • No histopathological changes reported (1,200) <u>NOAEL = 450 mg/kg bw/day</u>	Omara and Blakley (1993)
Rat (Sprague-Dawley) 40 M/group	2.84, 5.69, or 11.54 mg iron/kg bw/day	31 or 61 days	• NSD in body weight, food consumption, hematology parameters • ↓ plasma sodium concentration at 31 and 61 days (dose-dependent) • ↓ plasma total iron binding capacity at 61 days (dose-dependent) • ↑ iron concentration in the spleen after 31 days and liver and kidney after 61 days (dose-dependent) • No toxicologically significant gross or microscopic abnormalities <u>NOAEL = 11.54 mg iron/kg bw/day</u>	Appel *et al.* (2001)
Developmental Toxicity				
Rat (Wistar) F[d]	0 or 110 mg iron/kg bw/day	NR	• NSD in mean number of resorption sites, number of fetuses, or fetal dry weight • ↓ fetal wet weight in adequate-zinc diet group	Fairweather-Tait *et al.* (1984)
Mice (CD-1) F	Up to 160 mg/kg bw/day	GD 6 to GD 16	• No maternal toxicity or teratogenicity	FDRLI (1974)

Table 6.3.3-1 Key Findings of Toxicity Studies on Ferrous Sulfate (Adapted from GRN 271)[a]

Test Species	Dose/Concentration	Duration	Results[b]	Reference
Rat (Wistar) F	Up to 200 mg/kg bw/day	GD 6 to GD 15	• No maternal toxicity or teratogenicity	FDRLI (1974)

ALP = alkaline phosphatase; ALT = alanine aminotransferase; bw = body weight; F = female; GD = gestational day; M= male; NR = not reported; NOAEL = no-observed-adverse-effects level; NSD = no significant differences.
[a] The results were adapted from the information as presented in GRN 271 (Nestlé USA, 2008).
[b] Reported findings were statistically significant from the control unless otherwise noted.
[c] Infant and young adult females.
[d] Animals were provided a low or adequate zinc diet before pregnancy.

6.2.6.3 Animal Studies on Other Iron Protein Chelates

A number of animal studies exists on other iron-casein chelates such as iron-β-casein and iron-β(1,25)-caseinate (Ait-oukhatar *et al.,* 1999, 2002;Pérès *et al.,* 1999; Bouhallab and Bougle, 2004). In general, these studies were conducted to investigate the rate of iron absorption compared to other inorganic iron sources (*e.g.,* ferrous sulfate, ferrous gluconate) and did not include any safety-related parameters. Although the test articles used in these studies were not directly extrapolatable to iron milk proteinate, nor did they utilize suitable study designs for use in risk assessment, therefore were no findings reported by the authors to suggest that milk protein chelates of iron are suitable or unsafe for use as a source of dietary iron for food fortification.

6.3 Allergenicity of Iron Milk Proteinate

The iron milk proteinate is produced with the use of casein or derivatives of casein; casein is a natural component of milk which is considered a major food allergen subject to labeling under the Food Allergen Labeling and Consumer Protection Act (FALCPA) as regulated by the FDA (U.S. FDA, 2004b).

The allergenicity of casein was considered through a search of the 3 major allergen databases: AllergenOnline[5], COMPARE[6], and WHO/IUIS Allergen Database[7]. A number of putative allergens related to casein were identified from cows (*Bos taurus*) and yaks (*Bos grunniens mutus*). The search results from the AllergenOnline database are summarized in Table 6.5-1 below. The search results of the COMPARE database were similar to those of AllergenOnline. The identified allergenic proteins have been demonstrated experimentally to contain IgE-binding activity *via* Western blot or enzyme-linked immunosorbent assay (ELISA) analysis and skin prick test, in addition to biological activity as measured by basophil activation, that are characteristics of allergenic proteins.

[5] AllergenOnline is an allergen protein database containing 2,129 peer-reviewed allergenic protein sequences (Version 19; released on February 10, 2019) that is curated by the Food Allergy Research and Resource Program (FARRP) of the University of Nebraska. The database is available at: http://www.allergenonline.org/
[6] The COMprehensive Protein Allergen REsource (COMPARE) database is a manually-curated allergenic protein database maintained by the Health and Environmental Sciences Institute (HESI). The COMPARE database contains about 2,081 allergenic proteins in total. The database is available at: http://db.comparedatabase.org/
[7] The WHO/IUIS allergen database contains 948 allergenic proteins and is maintained by the World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-Committee. The database is available at: http://www.allergen.org/index.php

Table 6.5-1 Allergenic Proteins Related to Casein from the AllergenOnline Database (Version 19)

Species	Common Name	Allergen	Allergenicity	Amino Acid Length	GI #
Bos grunniens mutus	Yak	Bos Bos d 11 beta casein	IgE plus basophil+ or SPT+	259	942073448
Bos taurus	Bovine	Bos Alpha-s1 casein	IgE plus basophil+ or SPT+	93	162650
Bos taurus	Bovine	Bos Alpha-s1 casein	IgE plus basophil+ or SPT+	214	162794
Bos taurus	Bovine	Bos Alpha-s1 casein	IgE plus basophil+ or SPT+	76	162927
Bos taurus	Bovine	Bos Alpha-s1 casein	IgE plus basophil+ or SPT+	214	30794348
Bos taurus	Bovine	Bos Alpha-s1 casein	IgE plus basophil+ or SPT+	205	159793197
Bos taurus	Bovine	Bos Alpha-s1 casein	IgE plus basophil+ or SPT+	172	159793201
Bos taurus	Bovine	Bos Alpha-s1 casein	IgE plus basophil+ or SPT+	129	159793217
Bos taurus	Bovine	Bos Bos d 11 beta casein	IgE plus basophil+ or SPT+	224	162797
Bos taurus	Bovine	Bos Bos d 11 beta casein	IgE plus basophil+ or SPT+	224	162805
Bos taurus	Bovine	Bos Bos d 11 beta casein	IgE plus basophil+ or SPT+	224	459292

A search of the WHO/IUIS Allergen database for “casein” identified 5 results, including Bos d 8 (caseins), Bos d 9 (alphaS1-casein), Bos d 10 (alphaS2-casein), Bos d 11 (beta-casein), and Bos d 12 (kappa-casein). These proteins have a molecular weight ranging from 19 to 30 kDa. The totality of evidence indicates that casein has the potential to elicit allergenic reactions in consumers. Products containing milk, and, as a result, casein, that are currently on the market are clearly labeled as containing milk such that consumers who have milk allergies are able to self-regulate and avoid. Since casein is a component of milk proteins, products containing casein is subject to labeling under FALCPA. Therefore, foods containing the iron milk proteinate will be clearly labelled as ‘containing milk’.

7.0 CONCLUSIONS AND BASIS FOR GRAS

The data and information summarized herein demonstrate that iron milk proteinate, as manufactured by Nestlé using cGMP and meeting appropriate food-grade specifications, is GRAS based on scientific procedures, under the conditions of intended use in foods and beverages as described herein.

PART 7. § 170.255 LIST OF SUPPORTING DATA AND INFORMATION

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Table of CFR Sections Referenced (Title 21—Food and Drugs)

Part	Section §	Section Title
101—Food labeling	101.9	Nutrition labeling of food
	101.12	Reference amounts customarily consumed per eating occasion
	101.13	Nutrient content claims-general principles
104—Nutritional quality guidelines for foods	104.20	Statement of purpose
109—Unavoidable contaminants in food for human consumption and food-packaging material	109.30	Tolerances for polychlorinated biphenyls (PCB's)
110—Current good manufacturing practice in manufacturing, packing, or holding human food	All sections	
170—Food Additives	170.30	Eligibility for classification as generally recognized as safe (GRAS)
182—Substances generally recognized as safe	182.1748	Sodium caseinate
184—Direct Food Substances Affirmed as Generally Recognized as Safe	All sections	
1240—Control of communicable diseases	1240.61	Mandatory pasteurization for all milk and milk products in final package form intended for direct human consumption

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18 December 2020

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Dear Mr. Kampmeyer:

Re: Responses to GRN 000959 for Iron Milk Proteinate

Please see below responses to the United States (U.S.) Food and Drug Administration (FDA)'s queries on GRAS Notice (GRN) No. 959 pertaining to iron milk proteinate.

Question 1. Please clarify whether iron milk proteinate is intended for use in infant formula or any products under the jurisdiction of the United States Department of Agriculture.

Response 1. Iron milk proteinate is not intended for use in infant formula or any food products under the jurisdiction of the USDA.

Question 2. You stated that background iron exposure was estimated using data from the National Health and Nutrition Examination Survey (NHANES III, 1988-1994). Please provide an updated dietary exposure assessment for the background and proposed uses using recent food consumption data.

Response 2.

An updated dietary intake assessment of iron from the background diet in the U.S. population was conducted using consumption data from the 2015-2016 National Health and Nutrition Examination Survey (NHANES). As iron milk proteinate is intended for use as a dietary source of iron in conventional food and beverage products in the U.S., and its uses will be fully substitutional to other iron ingredients currently on the U.S. marketplace, it was assumed that iron from iron milk protein is the sole source of iron in the diet (*i.e.,* all iron intake from the background diet is a result of iron milk proteinate consumption). As a result, iron milk proteinate intakes were calculated based on iron intake estimates from the background diet (using NHANES 2015-2016) and the average iron content of iron milk proteinate from proximate analyses (2.7%, see Table 2.4.1-1 of the GRAS notice). Corresponding intakes of phosphorous and casein from iron milk proteinate were also calculated based on the average content of phosphorus and protein from proximate analyses of iron milk proteinate, respectively (61% protein and 3.5% phosphorous, see Table 2.4.1-1 of the GRAS notice).

NHANES 2015-2016 dietary survey data were collected from individuals and households *via* 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) throughout all 4 seasons of the year (CDC,

2018a,b,c[1,2,3]; USDA, 2020[4]). Participants who completed the dietary intake data collection were also asked to complete a similar recall in which they documented the supplement products consumed within the previous 24 hours on 2 non-consecutive days (CDC, 2019a,b[5,6]). The amounts of individual ingredients present in the supplement were itemized and entered into the NHANES database, which allows the data to be incorporated into the dietary intake estimates (CDC, 2020[7]).

Nutrient values for foods and beverages consumed in NHANES 2015-2016 are available from the 2015-2016 United States Department of Agriculture's Food and Nutrient Database for Dietary Studies (FNDDS) (USDA, 2018). All food codes with an associated iron nutrient value were selected for the assessment, applying the associated iron value (expressed in mg/100 g) as the use level. Similarly, all dietary supplements codes identified as containing iron as an ingredient were selected for the assessment (CDC, 2020), applying the associated amount of iron per serving (expressed as mg/g in food equivalents, multiplied by 100 g) as the use level.

Consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer and used to generate estimates for the intake of iron by the U.S. population[8]. Sample weights were incorporated with NHANES data to compensate for the potential under-representation of intakes from specific populations and allow the data to be considered nationally representative (CDC, 2018a,b; USDA, 2020). Estimates for the daily intake of iron represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2015-2016; these average amounts comprised the distribution from which mean

[1] CDC (2018a). Dietary interview - individual foods, first day. In: *National Health and Nutrition Examination Survey (NHANES): 2015-2016 – Dietary Data*. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: https://wwwn.cdc.gov/nchs/nhanes/search/datapage.aspx?Component=Dietary&CycleBeginYear=2015 [Last updated: July 2018].

[2] CDC (2018b). Dietary interview - individual foods, second day. In: *National Health and Nutrition Examination Survey (NHANES): 2015-2016 – Dietary Data*. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: https://wwwn.cdc.gov/nchs/nhanes/search/datapage.aspx?Component=Dietary&CycleBeginYear=2015 [Last updated: July 2018].

[3] CDC (2018c). *National Health and Nutrition Examination Survey (NHANES): 2015-2016*. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx?BeginYear=2015 [NHANES Home Page last reviewed: October 30, 2018].

[4] USDA (2020). *What We Eat in America: National Health and Nutrition Examination Survey (NHANES): 2015-2016.* Riverdale (MD): U.S. Department of Agriculture (USDA). Available at: http://www.ars.usda.gov/Services/docs.htm?docid=13793#release [Last Modified: 7/1/2020].

[5] CDC (2019a). Dietary supplement use 24-hour - individual dietary supplements, first day. In: *National Health and Nutrition Examination Survey (NHANES): 2015-2016 – Dietary Data*. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: https://wwwn.cdc.gov/nchs/nhanes/search/datapage.aspx?Component=Dietary&CycleBeginYear=2015 [Last updated: September 2019].

[6] CDC (2019b). Dietary supplement use 24-hour - individual dietary supplements, second day. *National Health and Nutrition Examination Survey (NHANES): 2015-2016 – Dietary Data*. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: https://wwwn.cdc.gov/nchs/nhanes/search/datapage.aspx?Component=Dietary&CycleBeginYear=2015 [Last updated: September 2019].

[7] CDC (2020). Dietary supplement database - ingredient information. In: *National Health and Nutrition Examination Survey (NHANES): 2015-2016 – Dietary Data*. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: https://wwwn.cdc.gov/nchs/nhanes/search/datapage.aspx?Component=Dietary&CycleBeginYear=2015 [Last updated: August 2020].

[8] Statistical analysis and data management were conducted in DaDiet Software (Dazult Ltd., 2018 - http://dadiet.daanalysis.com). DaDiet Software is a web-based software tool that allows accurate estimate of exposure to nutrients and to substances added to foods, including contaminants, food additives and novel ingredients. The main input components are concentration (use level) data and food consumption data. Data sets are combined in the software to provide accurate and efficient exposure assessments.

and 90th percentile intake estimates were determined. Only the consumer-only intake estimates are discussed herein as these are more relevant to risk assessment. "Consumer-only" intake refers to the estimated intake of iron by only those individuals who reported consuming iron-containing foods or supplements on either Day 1 or Day 2 of the survey. Estimated daily intakes of iron from the background diet (food and supplements) using NHANES 2015-2016 are reported for the same population groups as those for which Dietary Reference Intake (DRI) values have been established by the IOM (excluding pregnant and lactating women) (IOM, 2006[9]; see Table 3.3-1 of the GRAS notice), namely:

- Children, 1 to 3 years;
- Children, 4 to 8 years;
- Male pre-adolescents, 9 to 13 years;
- Male adolescents, 14 to 18 years;
- Male young adults, 19 to 30 years;
- Male adults, 31 to 50 years;
- Elderly males, 51 to 70 years;
- Very elderly males, ages 71 years and older;
- Female pre-adolescents, 9 to 13 years;
- Female adolescents, 14 to 18 years;
- Female young adults, 19 to 30 years;
- Female adults, 31 to 50 years;
- Elderly females, 51 to 70 years;
- Very elderly females, ages 71 years and older; and
- All individuals (ages 1 year and older, both gender groups combined).

Among all individuals (≥ 1 year of age), mean and 90th percentile intakes of iron from the background diet (food and supplements), assuming 100% replacement of iron in the diet with iron from iron milk proteinate, were determined to be 16.42 and 27.92 mg/person/day, respectively. Among individual population groups, mean and 90th percentile intakes of iron were highest in very elderly males 71 years of age and older, at 20.07 and 36.91 mg/person/day, respectively. In all population groups, the estimated daily intakes of iron at the mean and 90th percentile are below upper tolerable limit (UL) values for iron established by the IOM (see Table 3.3-1 of the GRAS notice).

Corresponding iron milk proteinate intakes among all individuals (≥ 1 year of age) at the mean and 90th percentile following the replacement of all iron in the diet with iron from iron milk proteinate were calculated at 608 and 1,034 mg/person/day, respectively. Highest mean and 90th percentile intakes calculated in very elderly males over 71 years of age and older were of 743 and 1,367 mg/person/day, respectively.

Phosphorous intakes from iron milk proteinate in this replacement scenario were calculated at 21.29 and 36.19 mg/person/day at the mean and 90th percentile among all individuals (≥ 1 year of age), respectively. Highest mean and 90th percentile intakes among very elderly males over 71 years of age and older were calculated at 26.02 and 47.85 mg/person/day, respectively. In all population groups, the estimated daily intakes of phosphorous from iron milk proteinate at the mean and 90th percentile are well below the UL established for phosphorous by the IOM (see Table 3.3-2 of the GRAS notice).

[9] IOM (2006). Phosphorus. In: *Dietary Reference Intakes: The Essential Guide to Nutrient Requirements*. (National Academy of Sciences/NAS, Institute of Medicine/IOM, Food and Nutrition Board/FNB). Washington (DC): National Academy Press (NAP), pp. 362-369, 1194-1244. Available at: http://books.nap.edu/catalog.php?record_id=11537.

Casein intakes from iron milk proteinate in this replacement scenario were calculated at 371 and 631 mg/person/day at the mean and 90th percentile among all individuals (≥ 1 year of age), respectively. Highest mean and 90th percentile intakes among very elderly males over 71 years of age and older were calculated at 453 and 834 mg/person/day, respectively.

Table 1 Consumer-Only Estimated Daily Intake of Iron from the Background Diet (Food and Supplements) and Corresponding Daily Intakes of Iron Milk Proteinate and Phosphorous and Casein from Iron Milk Proteinate in the U.S. by Population Group (2015-2016 NHANES Data)

Life Stage Group		Estimated Intake of Iron from Food and Supplements (mg/person/day)		Estimated Intake of Iron Milk Proteinate (mg/person/day)[a]		Estimated Intake of Phosphorous from Iron Milk Proteinate (mg/person/day)[b]		Estimated Intake of Casein from Iron Milk Proteinate (mg/person/day)[c]	
		Mean	P90	Mean	P90	Mean	P90	Mean	P90
Children	1 to 3 years	10.18	17.39	377	644	13.20	22.54	230	393
	4 to 8 years	13.49	20.63	500	764	17.49	26.74	305	466
Males	9 to 13 years	16.06	24.51	595	908	20.82	31.77	363	554
	14 to 18 years	17.95	28.63	665	1,060	23.27	37.11	406	647
	19 to 30 years	17.35	30.30	643	1,122	22.49	39.28	392	685
	31 to 50 years	17.27	27.08	640	1,003	22.39	35.10	390	612
	51 to 70 years	17.70	27.67	656	1,025	22.94	35.87	400	625
	≥71 years	20.07	36.91	743	1,367	26.02	47.85	453	834
Females	9 to 13 years	14.68	24.92	544	923	19.03	32.30	332	563
	14 to 18 years	13.63	21.95	505	813	17.67	28.45	308	496
	19 to 30 years	16.04	28.11	594	1,041	20.79	36.44	362	635
	31 to 50 years	17.05	33.27	631	1,232	22.10	43.13	385	752
	51 to 70 years	15.81	27.38	586	1,014	20.49	35.49	357	619
	≥71 years	18.18	31.81	673	1,178	23.57	41.24	411	719
All individuals	≥1 year	16.42	27.92	608	1,034	21.29	36.19	371	631

NHANES = National Health and Nutrition Examination Survey; P90 = 90th percentile U.S. = United States.

[a] Iron comprises 2.7% of iron milk proteinate on average (see proximate analyses in Table 2.4.1-1 of the notice).

[b] Phosphorous comprises 3.5% of iron milk proteinate on average (see proximate analyses in Table 2.4.1-1 of the notice).

[c] Iron milk proteinate is a complex of iron-casein-phosphate; protein comprises 61% of iron milk proteinate on average (see proximate analyses in Table 2.4.1-1 of the notice).

Question 3. Please provide specifications along with corresponding batch analysis data for three non-consecutive lots for mercury.

Response 3. The results of analysis of the same 6 non-consecutive lots of iron milk proteinate as presented in the GRAS notice for mercury using ICP-MS are presented in Table 1 below.

Table 1 Mercury Analyses of 6 Non-Consecutive Lots of Iron Milk Proteinate

Heavy Metal	Specification Limit	Manufacturing Lot					
		17 FP201	19 FP202	23 FP203	18 FP301	20 FP302	24 FP303
Mercury (mg/kg)	N/A	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2

Question 4. We note that iron milk proteinate is intended for use in conventional food and beverage products in the U.S. Please confirm the specific food categories with corresponding use levels in which iron milk proteinate is intended to be used.

Response 4. Iron milk proteinate is intended for use as a dietary source of iron, and will act as a direct replacement for other iron sources in existing categories of fortified foods in the U.S. (*i.e.,* will be used in accordance with the FDA's Fortification Policy under 21 CFR 104.20 (U.S. FDA, 2020)[10]). Iron milk proteinate will be used in the same food categories as other iron fortificants at levels based on good manufacturing practice. The proposed uses are consistent with those described for sodium ferrous citrate under GRN 441 which received no questions from the FDA on May 10, 2013 (U.S. FDA, 2013)[11].

Question 5. Please indicate if the analytical methods used for all analyses are validated and fit for purpose.

Response 5. All analyses were performed in-house using validated methods or by an accredited third-party laboratory (Hill Laboratories, New Zealand) using internationally recognized methods (e.g. AOAC) or validated for their purposes.

Question 6. Pages 34-35 of the notice state:

> *In a series of short-term, subchronic, and chronic toxicity studies conducted with various phosphates and polyphosphates, the only reported significant adverse effect of excessive phosphate consumption is calcification of the kidney and tubular nephropathy. In human clinical studies, impairment of renal function was reported following consumption of doses up to 4,800 mg/day (d), equivalent to 68.6 mg/kg body weight (bw)/d for a 70-kg individual, while no such kidney effects were reported following daily consumption of 2,000 mg phosphorous, equivalent to 28.6 mg/kg bw/d.*

Please clarify whether these are the conclusions of EFSA (2019), and if not, please provide reference(s) for the above statement.

[10] U.S. FDA (2020). Part 104—Nutritional quality guidelines for foods. §104.20—Statement of purpose. In: *U.S. Code of Federal Regulations (CFR). Title 21: Food and Drugs*. (U.S. Food and Drug Administration). Washington (DC): U.S. Government Printing Office (GPO). Available at: https://www.govinfo.gov/app/collection/cfr/.

[11] U.S. FDA (2013). *Agency Response Letter GRAS Notice No. GRN 441 [Sodium ferrous citrate, Tokyo, Japan: Eisai Food and Chemical Co., Ltd.]*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN), Office of Food Additive Safety. Available at: http://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&id=441 [May 10, 2013 - FDA response - no questions; some uses may require a color additive listing].

Response 6. These are the conclusions of EFSA (2019[12]) as presented in page 3.

Sincerely,

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Dairy Strategic Business Unit
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[12] EFSA (2019). Scientific Opinion on the re-evaluation of phosphoric acid–phosphates–di-, tri- and polyphosphates (E 338–341, E 343, E 450–452) as food additives and the safety of proposed extension of use. (EFSA Panel on Food Additives and Flavourings/FAF) (Question nos EFSA-Q-2011-00532, EFSA-Q-2011-00533, EFSA-Q-2011-00534, EFSA-Q-2011-00535, EFSA-Q-2011-00536, … EFSA-Q-2018-00597, adopted: 4 June 2019 by European Food Safety Authority). EFSA J 17(6):5674 [156 pp.]. DOI:10.2903/j.efsa.2019.5674. Available at: https://www.efsa.europa.eu/en/efsajournal/pub/5674.