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**EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA); Turck, Dominique; Heinonen, Marina**

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# Safety of iron milk proteinate as a novel food pursuant to Regulation (EU) 2015/2283 and bioavailability of iron from this source in the context of Directive 2002/46/EC

EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA), Dominique Turck, Jacqueline Castenmiller, Stefaan De Henauw, Karen Ildico Hirsch-Ernst, Alexandre Maciuk, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Carmen Pelaez, Kristina Pentieva, Alfonso Siani, Frank Thies, Sophia Tsabouri, Marco Vinceti, Francesco Cubadda, Thomas Frenzel, Marina Heinonen, Miguel Prieto Maradona, Rosangela Marchelli, Monika Neuhäuser-Berthold, Morten Poulsen, Josef Rudolf Schlatter, Henk van Loveren, Reinhard Ackerl and Helle Katrine Knutsen

## Abstract

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on iron milk proteinate as a novel food (NF) pursuant to Regulation (EU) 2015/2283 and to address the bioavailability of iron from this source in the context of Directive 2002/46/EC. The NF is a complex of iron, casein and phosphate, which is produced from iron salts (i.e. ferric chloride or ferric sulfate), sodium caseinate and potassium orthophosphate. The NF is proposed by the applicant to be used as a source of iron, of which the NF contains 2–4%. The applicant intends to market the NF as an ingredient in a number of food categories; in food supplements, in total diet replacement for weight control and in foods for special medical purposes. The Panel considers that, taking into account the composition of the NF and the proposed conditions of use, consumption of the NF is not nutritionally disadvantageous. The studies provided for ADME and bioavailability indicate that iron from the NF is bioavailable. Overall, the evidence indicates that upon ingestion the NF undergoes digestion into small peptides to yield iron-bound caseinophosphopeptides that are normal constituents of the human diet, and that the iron from the NF does not bypass the homeostatic control of iron as a nutrient. The Panel concludes that the NF, iron milk proteinate, is safe under the proposed conditions of use. The Panel also concludes that the NF is a source from which iron is bioavailable.

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**Keywords:** iron milk proteinate, novel food, nutrient source, iron, bioavailability, safety

**Requestor:** European Commission

**Question number:** EFSA-Q-2020-00492

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**Panel members:** Dominique Turck, Jacqueline Castenmiller, Stefaan De Henauw, Karen Ildico Hirsch-Ernst, Helle Katrine Knutsen, Alexandre Maciuk, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Carmen Pelaez, Kristina Pentieva, Alfonso Siani, Frank Thies, Sophia Tsabouri and Marco Vinceti.

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# 1. Introduction

## 1.1. Background and Terms of Reference as provided by the European Commission

On 15 June 2020, the company Société des Produits Nestlé S.A. submitted a request to the Commission in accordance with Article 10 of Regulation (EU) No 2015/2283 to place on the EU market iron milk proteinate.

Iron milk proteinate is intended to be used in a number of foods, in food supplements as defined in Directive 2002/46/EC, and in foods for special medical purposes as defined by Regulation (EU) 609/2013.

In accordance with Article 10(3) of Regulation (EU) 2015/2283, the European Commission asks the European Food Safety Authority to provide a scientific opinion on iron milk proteinate. In addition, as iron milk proteinate is also a new source of iron, the opinion should also address the bioavailability of iron from this source in the context of Directive 2002/46/EC of the European Parliament and of the Council laying down requirements for food supplements.[1]

## 1.2. Information on existing evaluations and authorisations

In 2015, the EFSA NDA Panel set dietary reference values for iron (EFSA NDA Panel, 2015). No Tolerable Upper Intake Level (UL) has been set for iron by the Scientific Committee on Food (SCF) or EFSA (EFSA, 2004). For further information see Section 3.9.

# 2. Data and Methodologies

## 2.1. Data

The safety assessment of this NF is based on data supplied in the application and information submitted by the applicant following EFSA's requests for supplementary information.

Administrative and scientific requirements for NF applications referred to in Article 10 of Regulation (EU) 2015/2283 are listed in Commission Implementing Regulation (EU) 2017/2469[2].

A common and structured format on the presentation of NF applications is described in the EFSA guidance on the preparation and presentation of a NF application (EFSA NDA Panel, 2016). As indicated in this guidance, it is the duty of the applicant to provide all of the available (proprietary, confidential and published) scientific data, (including both data in favour and not in favour) that are pertinent to the safety of the NF.

This NF application includes a request for protection of proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283. The data requested by the applicant to be protected comprise: acute oral toxicity study (Suman, 2019, unpublished), certificates of analyses, lab accreditations, literature search and intake assessment as performed by the applicant, *in vitro* digestibility report ('Annex G'), *in vitro* study (Cornell University, 2021, unpublished), *in vitro* study (Sabatier et al., 2020), human study (Henare et al., 2019).

## 2.2. Methodologies

The assessment follows the methodology set out in the EFSA guidance on NF applications (EFSA NDA Panel, 2016) and the principles described in the relevant existing guidance documents from the EFSA Scientific Committee. The legal provisions for the assessment are laid down in Article 11 of Regulation (EU) 2015/2283 and in Article 7 of Commission Implementing Regulation (EU) 2017/2469.

This assessment concerns only the risks that might be associated with consumption of the NF under the proposed conditions of use, and is not an assessment of the efficacy of the NF with regard to any (claimed) benefit.

[1] Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements. OJ L 183, 12.7.2002, p. 51.

[2] Commission Implementing Regulation (EU) 2017/2469 of 20 December 2017 laying down administrative and scientific requirements for applications referred to in Article 10 of Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. OJ L 351, 30.12.2017, pp. 64–71.



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The evaluation of bioavailability of the nutrient (iron) from the source (iron milk proteinate (IMP)) was conducted in line with the principles contained in the 'Guidance on safety evaluation of sources of nutrients and bioavailability of nutrient from the sources' (EFSA ANS Panel, 2018).

# 3. Assessment

## 3.1. Introduction

The NF which is the subject of the application is IMP, which is a complex of iron, casein and phosphate. The NF is proposed to be used in a number of foods, food supplements and foods for special medical purposes as a source of iron.

The NF falls under the following category, as defined in Art. 3 of Reg. (EU) 2015/2283: ix vitamins, minerals and other substances used in accordance with Directive 2002/46/EC, Regulation (EC) No 1925/2006 or Regulation (EU) No 609/2013.

## 3.2. Identity of the NF

The NF, IMP, is a creamy/beige odourless powder that is highly dispersible in water. It is constituted of casein complexed to ferric ions ($Fe^{3+}$) through phosphoserine residues and stabilised by phosphate. Experimental evidence was provided by the applicant to demonstrate that the NF is an iron-casein-phosphate complex, besides literature reporting the formation of a complex involving iron, casein and inorganic phosphorous (Gaucheron et al., 1997; Miquel et al., 2005; Raouche et al., 2009). The composition of the proteins in the IMP complex was determined by size exclusion chromatography (SEC) and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). It was demonstrated that all the major casein phosphoproteins (αS1-, αS2-, β- and k-casein) are present in the main fraction of the iron-added sodium caseinate solution containing orthophosphate. The binding of iron to casein was investigated using $^{31}$P-NMR (Mittal et al., 2016). The data presented show that, upon addition of $Fe^{3+}$ ions to a solution containing casein and orthophosphate, the $Fe^{3+}$ ions form a complex with the phosphate residues of casein proteins and inorganic phosphate via electrostatic interactions and coordinate bonds. The binding of iron to casein remains unaffected by a reduction in pH down to approximately pH 5. In addition, solubility experiments were performed and centrifugal ultrafiltration was applied. The absence of red precipitates and iron in the permeate suggested that all the iron present in the system (at pH 6.8) was present as bound iron in the form of an iron-casein-phosphate complex.

The applicant indicated the following synonyms/trade names for the NF: iron-casein-phosphate complex, ferric caseinate, FerriPro.

## 3.3. Production process

According to the information provided, the NF is produced following current Good Manufacturing Practice (cGMP) and Hazard Analysis Critical Control Points (HACCP) principles.

The applicant provided a list of the raw materials used in the production process, along with their certificates of analyses.

IMP is manufactured by dissolving ferric iron salts (i.e. ferric chloride or ferric sulfate) in a caseinate solution (derived from sodium caseinate powder, complying with the purity requirements for 'edible caseinates' as defined in Annex II of Directive (EU) 2015/2203[3]) in the presence of potassium orthophosphate. The pH of the solution is maintained at near neutral to ensure that the proteins do not precipitate from solution. The solution is stirred, pasteurised, concentrated and spray-dried into a powder.

The Panel considers that the production process is sufficiently described and does not raise safety concerns.

## 3.4. Compositional data

The NF is primarily composed of protein (55–65%) and minerals, including iron (2.3–3.4%), phosphorus (3.1–4.0%) and potassium (7.3–7.7%) (range of values of the batch testing of the NF).

[3] Directive (EU) 2015/2203 of the European Parliament and of the Council of 25 November 2015 on the approximation of the laws of the Member States relating to caseins and caseinates intended for human consumption and repealing Council Directive 83/417/EEC. OJ L 314, 1.12.2015, pp. 1–9.



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In order to confirm that the manufacturing process is reproducible and adequate to produce on a commercial scale a product with certain characteristics, the applicant provided analytical information on proximates, lactose, pH, microbiological parameters and a number of minerals including iron for five batches of the NF produced with ferric sulfate (Table 1).

**Table 1:** Batch-to-batch analysis of the NF (produced with ferric sulfate)

| Parameter (unit) | Batch number | | | | | Method of analysis |
|---|---|---|---|---|---|---|
| | #1 | #2 | #3 | #4 | #5 | |
| Protein (%) (N × 6.38)(1) | 61 | 55 | 63 | 63 | 62 | Dumas combustion AOAC 992.15, 19th edition |
| Ash (%) | 32 | 39 | 31 | 31 | 31 | Ignition; AOAC 942.05, 20th edition |
| Moisture (%) | 2.9 | 2.6 | 3.2 | 3.3 | 3.3 | Drying; AOAC 945.15, 20th edition |
| Fat (%) | 0.6 | 0.4 | 0.3 | 0.2 | < 0.1 | Acid hydrolysis AOAC 948.15 |
| Lactose monohydrate (%) | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | In-house method |
| Iron (%) | 2.7 | 3.4 | 2.7 | 2.3 | 2.6 | ICP-OES |
| Phosphorus (%) | 3.5 | 4.0 | 3.5 | 3.1 | 3.5 | ICP-OES |
| Calcium (%) | 0.047 | 0.044 | 0.048 | 0.043 | 0.048 | ICP-OES |
| Sodium (%) | 2.3 | 2.7 | 2.2 | 2.0 | 2.2 | ICP-OES |
| pH | 6.9 | 6.8 | 6.9 | 6.9 | 6.9 | pH meter(2) |
| Lead (mg/kg) | < 0.10 | < 0.10 | < 0.10 | < 0.10 | < 0.10 | ICP-MS |
| Aerobic plate count (cells/g) | 110 | 89 | 21 | < 10 | 10 | RLP Official Test 11.5.3, 11.6.7, 11.8.6, 31.1.(3) |
| Total coliforms (cells/g) | < 10 | < 10 | < 10 | < 10 | < 10 | RLP Official Test 31.6(4) |
| Yeast and moulds (cells/g) | < 10 | < 10 | < 10 | < 10 | < 10 | RLP Official Test 31.16(5) |
| *Escherichia coli* (in 25 g) | not detected | not detected | not detected | not detected | not detected | In-house (APHA 9.93 modified) 5th ed. |
| *Staphylococcus aureus* (in 1 g) | not detected | not detected | not detected | not detected | not detected | APHA 39 5th ed. (modified) |
| *Salmonella* (in 25 g) | not detected | not detected | not detected | not detected | not detected | Qualitative real-time PCR |

AOAC: Association of Official Analytical Collaborations; APHA: American Public Health Association; ICP-MS: inductively coupled plasma mass spectrometry; ICP-OES: inductively coupled plasma-optical emission spectroscopy; PCR: polymerase chain reaction; RLP: Recognised Laboratory Programme.
(1): 6.38 represents a nitrogen conversion factor commonly used for milk protein.
(2): Sample diluted 1 part to 100 parts of water.
(3): Automated Most probable number (MPN) count on TEMPO AC, incubated at 30°C for 22–28 h. bioMérieux, TEMPO.
(4): Automated MPN count on TEMPO CC, incubated at 35°C for 22–27 h. bioMérieux, TEMPO.
(5): Automated MPN count on TEMPO YM, incubated at 25°C for 72–76 h. bioMérieux, TEMPO.

For six further lots of the NF produced with ferric chloride, the applicant submitted analyses for protein, iron, a number of additional metals and aflatoxin M1 (Table 2).

**Table 2:** Analysis of protein, iron, further metals and aflatoxin M1 in the NF (produced with ferric chloride)

| Parameter (unit) | Batch number | | | | | |
|---|---|---|---|---|---|---|
| | #6 | #7 | #8 | #9 | #10 | #11 |
| Protein (%) (N × 6.38) | 65 | 65 | 65 | 63 | 63 | 62 |
| Iron (%) | 2.6 | 2.5 | 2.6 | 2.5 | 2.5 | 2.4 |
| Cadmium (mg/kg) | < 0.08 | < 0.08 | < 0.08 | < 0.08 | < 0.08 | < 0.08 |



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| Parameter (unit) | Batch number | | | | | |
|---|---|---|---|---|---|---|
| | #6 | #7 | #8 | #9 | #10 | #11 |
| Copper (mg/kg) | 3.3 | 2.9 | 3.0 | 2.6 | 2.7 | 6.5 |
| Lead (mg/kg) | < 0.2 | < 0.2 | < 0.2 | < 0.2 | < 0.2 | < 0.2 |
| Mercury (mg/kg) | < 0.2 | < 0.2 | < 0.2 | < 0.2 | < 0.2 | < 0.2 |
| Potassium (g/100 g) | 7.7 | 7.6 | 7.7 | 7.3 | 7.5 | 7.4 |
| Aflatoxin M1 (μg/kg) | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 |

The applicant used transmission electron microscopy combined with energy-dispersive X-ray scattering (TEM-EDS) to show the presence of carbon, nitrogen, oxygen, iron and phosphorus in the NF.

The applicant also provided an analysis of the particle size distribution by dynamic light scattering (DLS) of the NF (spray dried powder dispersed in water), which showed the presence of entities in the nano size range.

The applicant used transmission electron microscopy (TEM) to determine the shape of the NF particles. According to the applicant, the NF particles have a dynamic shape that is diffuse and amorphous with no clear boundaries or defined shape. The applicant stated that the macromolecular structure of the NF is formed through self-assembly of protein molecules through hydrophobic, electrostatic, and ion-induced dipole interactions, and thus, the NF may be considered a 'soft' and hydrated material that changes shape and size depending on the environmental and solution conditions, without distinct or defined boundaries.

In summary, the applicant provided evidence to show that the NF is an iron-casein-phosphate complex and, based on the available knowledge, this complex is likely to exist in the form of soft nano-sized structures, which is consistent with the results by DLS submitted by the applicant.

The applicant stated that the manufacturing process for the NF does not include the use of any material that would introduce a source of mycotoxins or other secondary metabolites into the final product. To support this statement, the applicant provided analyses (by LC–MS/MS) of six independently produced lots of the NF for trichothecenes, zearalenone, fumonisins, ochratoxin A, aflatoxin M1 and sum of aflatoxins, which were all below the limits of detection, which the Panel considered sufficiently low.

Information was provided on the accreditation of the laboratories that conducted the analyses presented in the application.

The Panel considers that the information provided on the composition is sufficient for characterising the NF.

### 3.4.1. Stability

The applicant performed stability tests with five independently produced batches of the NF (the same as those given in Table 1). The tests were carried out at 30°C and at 37°C (at a relative humidity (RH) of 75%) for up to 12 months. The samples were kept in foil laminate resistant to light, moisture and air. The batches stored at 30°C were analysed for protein, moisture, water activity, iron, free iron, phosphorus, sodium, potassium, particle size and colour. The batches that were stored at 37°C were tested for iron, phosphorus, sodium, potassium and colour. There were no relevant changes observed during the storage. No information was provided on microbial counts. However, given the rather low water activity as measured in the samples over time (ranging from 0.093 to 0.119 at baseline and from 0.111 to 0.141 at 12 months) and the low numbers of aerobic plate count observed in the batch testing, the Panel considers that microbial growth is not expected during storage of the NF.

In another stability study, two batches of the NF were stored for 6 months at 20°C (RH 22–30%) and at 37°C (RH 23–34%). The batches were sealed in foil laminates that were impervious to light, moisture and air. Samples were drawn every month and physicochemical properties were measured, including water activity, which remained stable over the 6-month testing period.

The applicant also conducted studies assessing the sensory stability of the NF when added to milk powder or to a non-dairy ready-to-drink (RTD) beverage both containing high-DHA fish oil. The findings from both these studies suggest that the NF would not produce undesirable off-flavours in food products rich in unsaturated fats.

The Panel considers that the data provided sufficient information with respect to the stability of the NF for up to 12 months.



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## 3.5. Specifications

The specifications of the NF are indicated in Table 3.

**Table 3:** Specifications of the NF

| **Description:** A creamy or beige, odourless powder with a neutral taste manufactured using ferric salts (ferric sulfate or ferric chloride) and sodium caseinate. | |
|---|---|
| **Parameter (unit)** | **Specifications** |
| Protein (%) | 50–65 |
| Ash (%) | 20–40 |
| Moisture (%) | < 8 |
| Fat (%) | < 1 |
| Iron (%) | 2–4 |
| Potassium (%) | 5–15 |
| Phosphorus (%) | 2–6 |
| Sodium (%) | < 4 |
| Aflatoxin M1 (mg/kg) | < 0.02 |
| **Heavy metals** | |
| Lead (mg/kg) | < 0.5 |
| Arsenic (mg/kg) | < 1 |
| Cadmium (mg/kg) | < 0.5 |
| Mercury (mg/kg) | < 0.1 |
| **Microbiological** | |
| Aerobic plate count (CFU/g) | < 1,000 |
| Yeasts and moulds (CFU/g) | < 10 |
| Coliforms (CFU/g) | < 10 |
| *Escherichia coli* (CFU/g) | < 10 |
| *Salmonella* spp. (in 25 g) | not detected |
| *Staphylococcus aureus* (in 1 g) | not detected |

CFU: colony forming units.

Based on the results of the batch testing and the production process, the Panel considers that the aerobic plate count in the NF should be set at < 1,000 CFU/g (instead of < 10,000 CFU/g as proposed by the applicant).

The Panel considers that the information provided on the specifications of the NF is sufficient and does not raise safety concerns.

## 3.6. History of use of the NF and/or of its source

There is no history of use of the NF.

Casein and caseinates have an extensive history of use as they are naturally occurring proteins in milk.

In the EU, a number of different forms of iron (ferric or ferrous) are permitted for use in foods (Annex II of Regulation (EC) No 1925/2006[4]), food supplements (Annex II of Directive 2002/46/EC[5]) and foods for special groups (Annex of Regulation (EC) No 609/2013[6]).

[4] Regulation (EC) No 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods. OJ L 404, 30.12.2006, pp. 26–38.

[5] Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements. OJ L 183, 12.7.2002, pp. 51–57.

[6] Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control. OJ L 181, 29.6.2013, pp. 35–56.



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## 3.7. Proposed uses and use levels and anticipated intake

### 3.7.1. Target population

The applicant indicated that the NF is intended for use by all age groups over 4 years of age, including those consuming diets based on foods for special medical purposes (FSMPs).

As the NF is intended to be used as an ingredient in standard food categories, it cannot be excluded that the NF would also be consumed by other groups of the population. Therefore, the safety data and the exposure assessment shall cover all population groups (Commission Implementing Regulation (EU) 2017/2469, article 5(6)).

### 3.7.2. Proposed uses and use levels

The applicant informed that the NF is intended to be used as an ingredient for iron fortification in a number of food and beverage products in the EU, with the aim to provide iron at levels of 30% of the Nutrient Reference Value (NRV) for this nutrient in adults (14 mg/day (Regulation (EU) No 1169/2011[7])) per serving, equating to 4.2 mg iron. Using standard portion sizes and reconstitution factors (where applicable) the applicant proposed maximum use levels of iron from the NF in the food categories as indicated in Table 4. The applicant also clarified that the amount of the NF will be adjusted, depending on the iron content of the batch being used, in order to provide the required amount of iron in a given food category. These food categories, defined using the FoodEx2 hierarchy, and the maximum addition of iron from the NF, are reported in Table 4.

**Table 4:** Food categories and maximum use levels of iron from the NF as proposed by the applicant

| FoodEx2 level | FoodEx2 code | Food category | Max use level of iron from the NF (mg/100 g)(1) |
|---|---|---|---|
| L3 | A02PH | Milk and dairy powders | 19(2) (equivalent to 2.1 mg/100 g RTE) |
| L4 | A03GB | Isotonic and sport drinks | 1.7 |
| L4 | A03HH | Cocoa beverage preparation, powder | 16(3) (equivalent to 1.6 mg/100 g RTE) |
| L4 | A03GT | Malt coffee ingredient | 21(4) (equivalent to 1.6 mg/100 g RTE) |
| L3 | A00EY | Cereal bars | 14 |
| L5 | A007R | Asian-style noodles other than glass noodles | 1.5 |
| L3 | A043F | Stock cubes or granulate (bouillon base) | 95(5) (equivalent to 1.9 mg/100 g RTE) |
| L4 | A03RV | Single meal replacement for weight reduction | 2.4 |

NF: novel food; RTE: ready-to-eat.
Dilution factors for powders were obtained from EFSA (2018): https://zenodo.org/record/1256085#.XJqSgOSQxFo. These were used to determine the use level of iron from the NF for reconstituted food and beverage products, as consumed.
(1): Each batch of the NF should be analysed for its iron content. The addition of the NF as an ingredient is based on a maximum amount of iron in each food category.
(2): Reconstitution factor of 9 applied in the dietary exposure assessment.
(3): Reconstitution factor of 10 applied in the dietary exposure assessment.
(4): Reconstitution factor of 13 applied in the dietary exposure assessment.
(5): Reconstitution factor of 50 applied in the dietary exposure assessment.

The applicant also intends to market the NF for use in food supplements, at a maximum dose of 14 mg iron from the NF/day, excluding infants and young children. The Panel considers that the highest dose in food supplements is to be defined in accordance with the equivalent maximum amount of iron supplementation permitted at the national level.

Another proposed use for the NF is in total diet replacement for weight control as defined under Regulation (EU) No 609/2013, at a maximum use level of 4.7 mg iron from the NF per meal.

Furthermore, the applicant proposed the NF for use in FSMPs as defined in Regulation (EU) No 609/2013, excluding foods for infants and young children. The applicant informed that the level for use in FSMPs will be in accordance with the particular nutritional requirements of the persons for whom the products will be intended.

[7] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers [. . .]. OJ L 304, 22.11.2011, pp. 18–63.



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The applicant proposed that food supplements and foods containing the NF be labelled, indicating the proposed maximum daily dose for the NF and the information that foods containing the NF and food supplements should not be consumed on the same day.

### 3.7.3. Anticipated intake of the NF

Based on the maximum proposed use levels of iron from the NF as indicated in Table 4 and considering the lower specification limits of iron content (i.e. 2% (Table 3)) in the NF, the highest amounts of the NF that might potentially be added to arrive to the maximum iron levels in the proposed food categories were calculated (Table 5).

**Table 5:** Food categories and maximum potential use levels of the NF, considering an iron concentration of 2% in the NF (i.e. lower specification limit for iron)

| FoodEx2 level | FoodEx2 code | Food category | Max. use level of the NF(1) (mg NF/100 g) |
|---|---|---|---|
| L3 | A02PH | Milk and dairy powders | 950 |
| L4 | A03GB | Isotonic and sport drinks | 85 |
| L4 | A03HH | Cocoa beverage preparation, powder | 800 |
| L4 | A03GT | Malt coffee ingredient | 1,050 |
| L3 | A00EY | Cereal bars | 700 |
| L5 | A007R | Asian-style noodles other than glass noodles | 75 |
| L3 | A043F | Stock cubes or granulate (bouillon base) | 4,750 |
| L4 | A03RV | Single meal replacement for weight reduction | 120 |

NF: novel food.
(1): Considering an iron concentration of 2% in the NF, which is the lower limit according to the specifications (see Section 3.5).

Based on the highest amounts of the NF that might potentially be added (Table 5), EFSA performed an intake assessment of the anticipated daily intake of the NF, using individual data from the EFSA Comprehensive European Food Consumption Database (EFSA, 2011). The lowest and highest mean and 95th percentile anticipated daily intakes of the NF (in mg/day), among the EU dietary surveys, are presented in Table 6.

**Table 6:** Intake estimate of the NF (in mg/day) as an ingredient in the intended food categories

| Population group | Age (years) | Mean intake (mg/day) | | P95th intake (mg/day) | |
|---|---|---|---|---|---|
| | | Lowest(a) | Highest(a) | Lowest(b) | Highest(b) |
| Infants | < 1 | < 1 | 70 | < 1 | 430 |
| Young children(c) | 1 to < 3 | < 1 | 70 | < 1 | 320 |
| Other children | 3 to < 10 | < 1 | 60 | 5 | 190 |
| Adolescents | 10 to < 18 | < 1 | 70 | 7 | 230 |
| Adults(d) | ≥ 18 | < 1 | 60 | 4 | 250 |

NF: novel food.
(a): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 03/06/2022. The lowest and the highest averages observed among all EU surveys are reported in these columns. Owing to an ongoing revision, the data from Latvia for the food category stock cubes (A043F) were not considered in the intake assessment.
(b): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 03/06/2022. The lowest and the highest P95th observed among all EU surveys are reported in these columns (P95th based on less than 60 individuals are not considered). Owing to an ongoing revision, the data from Latvia for the food category stock cubes (A043F) were not considered in the intake assessment.
(c): Referred to as 'toddlers' in the EFSA Comprehensive European Food Consumption Database (EFSA, 2011).
(d): Includes elderly, very elderly, pregnant and lactating women.

### 3.7.4. Anticipated intake of iron from the NF used as a food ingredient

EFSA performed an intake assessment of the anticipated daily intake of iron from the NF based on the applicant's proposed uses and maximum proposed use levels of iron (Table 4), using individual data from the EFSA Comprehensive European Food Consumption Database (EFSA, 2011). The lowest and highest mean and 95th percentile anticipated daily intakes of iron from the NF (in mg/day), among the EU dietary surveys, are presented in Table 7.



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**Table 7:** Intake estimate of iron (mg/day) resulting from the use of the NF as an ingredient in the intended food categories at the maximum proposed use levels of iron

| Population group | Age (years) | Mean intake (mg/day) | | P95th intake (mg/day) | |
|---|---|---|---|---|---|
| | | Lowest[(a)] | Highest[(a)] | Lowest[(b)] | Highest[(b)] |
| Infants | < 1 | < 0.01 | 1.4 | < 0.01 | 8.7 |
| Young children[(c)] | 1 to < 3 | < 0.01 | 1.2 | < 0.01 | 6.5 |
| Other children | 3 to < 10 | < 0.1 | 1.2 | < 0.1 | 3.9 |
| Adolescents | 10 to < 18 | < 0.01 | 1.3 | < 0.01 | 4.6 |
| Adults[(d)] | ≥ 18 | < 0.01 | 0.3 | < 0.01 | 5.0 |

NF: novel food.
(a): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 03/06/2022. The lowest and the highest averages observed among all EU surveys are reported in these columns. Owing to an ongoing revision, the data from Latvia for the food category stock cubes (A043F) were not considered in the intake assessment.
(b): Intakes are assessed for all EU dietary surveys available in the food comprehensive database on 03/06/2022. The lowest and the highest P95th observed among all EU surveys are reported in these columns (P95th based on less than 60 individuals are not considered). Owing to an ongoing revision, the data from Latvia for the food category stock cubes (A043F) were not considered in the intake assessment.
(c): Referred to as 'toddlers' in the EFSA Comprehensive European Food Consumption Database (EFSA, 2011).
(d): Includes elderly, very elderly, pregnant and lactating women.

## 3.8. Absorption, distribution, metabolism and excretion (ADME)

Iron is inefficiently and variably absorbed, depending on dietary and host-related factors. In humans, its absorption occurs primarily in the duodenum. Homeostasis is mediated via the regulation of iron absorption, as there are no active pathways for excreting iron. In healthy individuals, the mucosal uptake and transfer of iron is inversely related to systemic serum ferritin concentrations (EFSA NDA Panel, 2015).

The applicant provided one *in vitro* digestibility study ('Annex G', unpublished), two *in vitro* studies with Caco-2 cells (Sabatier et al., 2020; Cornell University, 2021, unpublished) and one human study (Henare et al., 2019).

One *in vitro* study (Cornell University, 2021, unpublished) assessed the availability of iron from IMP when manufactured with casein from fresh milk, which the Panel considers not pertinent for the assessment of the NF.

The *in vitro* digestibility study ('Annex G', unpublished) was conducted with three batches of the NF (produced with ferric sulfate) using the protocol described by Minekus et al. (2014). Briefly, aqueous solutions of the NF (1% w/v) were mixed with simulated gastric fluid (pH 2.0) and pre-incubated at 37°C for 15 min. Next, digestion was initiated following addition of pepsin to the NF mixture. Digested samples were collected at 0, 0.5, 5, 10, 20 and 60 min. The digested samples were then diluted in reducing tricine SDS-PAGE sample buffer and the samples were analysed by SDS–PAGE. The samples were completely hydrolysed by 5 min and only small peptides were detected after 60 min of digestion. The rate of hydrolysis of casein fractions and their hydrolysis pattern in the NF were comparable to that of sodium caseinate. According to the applicant, the results of this study indicate that the caseinate component of the NF will be hydrolysed in a similar manner as sodium caseinate to yield peptides and amino acids, thus releasing iron and phosphate ions.

For the *in vitro* study by Sabatier et al. (2020) a simulated digestion coupled with a Caco-2 cell culture model was used in parallel with solubility and dissociation tests. Two iron-casein complexes (i.e. ICC1 and ICC2) were investigated in this study. The two complexes contained 2.8% and 2.9% iron, respectively. According to information provided by the applicant, the two complexes were produced with ferric chloride (ICC1) and ferric sulfate (ICC2), respectively.[8] Other than for the source of iron, the manufacturing process was similar (sodium caseinate was used as the source of casein, dipotassium hydrogen orthophosphate as the source of orthophosphate). There was a rapid solubilisation of iron from the ICC compounds in diluted acid (pH 1.7), i.e. > 75 ± 19.3% at 5 min and > 89 ± 0.3% at 90 min. This was similar to the kinetics of $FeSO_4$ (while the solubility of micronised FePP (ferric pyrophosphate) remained low and reached a maximum solubility of 37.6 ± 4.7% at 90 min). In order to evaluate whether the iron compounds dissociated from the proteins under acidic conditions, the

[8] The applicant informed that the sources of iron were not disclosed in the publication due to a pending patent application process at the time of publication.



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soluble fractions of the compounds were eluted through size-exclusion chromatography columns (at 30 min after the start of the experiment). While 86.6% of $FeSO_4$ was retrieved in the second eluate (corresponding to the free mineral fraction), only 10.7% of ICC1 and 20.5% of ICC2 were retrieved in this fraction. The percentages of iron appearing in the eluates corresponding to the protein fraction were 77.9%, 55.2% and 4.7% for ICC1, ICC2 and $FeSO_4$, respectively. This suggests that the majority of the iron from the ICCs remained bound to the protein under acidic conditions. Incubation of sodium caseinate, ICC1 and ICC2 with pepsin under simulated gastric conditions resulted in rapid hydrolysis of the proteins (as analysed by tricine SDS-PAGE). All casein fractions in the sodium caseinate sample showed significant hydrolysis within the first few minutes of incubation and no unhydrolysed casein fractions could be detected after 5 min of digestion. A similar pattern was observed for ICC1 and ICC2. Under this simulated gastric digestion also, the solubilities of $FeSO_4$ and both ICCs were measured. At 10 min, 98, 97 and 88% of the iron from $FeSO_4$, ICC1 and ICC2, respectively, was soluble.

The *in vitro* iron availability from both ICCs was evaluated in an *in vitro*-simulated digestion coupled with Caco-2 cells with and without ascorbic acid in water and in milk, in comparison with $FeSO_4$ (reference compound for iron bioavailability) and micronised FePP (commonly used for iron fortification of milk products). A simulated gastric digestion with pepsin (at pH = 2, 37°C for 1 h) was applied to mimic the gastric phase of the digestion, which was followed by a simulated intestinal digestion with pancreatin and bile (at pH = 7, 37°C for 2 h) to mimic the duodenal phase. This second step took place on a dialysis membrane placed above Caco-2 cell monolayers. Solubilised iron can diffuse through the membrane and be taken up by the cells, which, in response to the increase in intracellular iron concentrations, form ferritin. The formation of ferritin was measured as an indicator of iron uptake by the cells. In water in the absence of ascorbic acid, there was a higher iron uptake (measured as ferritin concentrations) by the Caco-2 cells from ICC1 (ICC2 not tested) than from $FeSO_4$ (and FePP). In milk in the absence of ascorbic acid, a similar effect was observed for ICC1 and ICC2 (and FePP). In water with the addition of ascorbic acid, iron uptake by the cells from the compounds was increased. In water with ascorbic acid the results corresponded to an *in vitro* bioavailability of iron from ICC1 relative to $FeSO_4$ of 121%. Also in milk the addition of ascorbic acid resulted in increases of iron uptake from all compounds tested. In milk in the presence of ascorbic acid, the *in vitro* bioavailability of iron from ICC1 and ICC2 relative to $FeSO_4$ amounted to 114 and 104%, respectively (FePP relative to $FeSO_4$: 36%).

The Panel considers that overall the evidence indicates that upon ingestion the NF would undergo digestion into small peptides to yield iron-bound caseinophosphopeptides that are normal constituents of the human diet.

### 3.8.1. Bioavailability of iron from the NF

The bioavailability of iron from the NF was investigated in a randomised, controlled crossover trial (Henare et al., 2019) in 21 healthy young women (age range 20–38 years; mean 25.2 ± 5.7) with normal iron status. Power calculations indicated that a sample size of n = 19 was needed in order to detect a 30% difference in iron absorption between the test compounds, using a two-sided superiority test (p-value $< 0.05$ and 90% power). The study participants consumed pasteurised whole milk containing 2.5 mg of isotopically labelled iron ($^{57}Fe$) from the NF (produced with labelled iron) or ferrous sulfate ($^{58}Fe$) (dual stable isotope technique). For the production of the NF, ferric chloride, sodium caseinate and dipotassium hydrogen orthophosphate were used as sources of iron, casein and phosphate, respectively. The batch of the tested NF contained 2.2% iron. 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 isotopes and calculation of the fractional iron absorption. The iron absorption from the NF and ferrous sulfate amounted to 3.4 ± 2% (geometric mean ± SD) and 3.9 ± 2.2%, respectively. The relative bioavailability of the NF to ferrous sulfate was approximately 87%. Iron absorption was negatively related to serum ferritin concentrations for both iron from the NF and iron from ferrous sulfate, with no statistically significant difference between the two sources of iron (i.e. the slopes of the linear regressions were not statistically significantly different between the two sources of iron), which suggests that iron absorption from the NF is regulated in a similar way as iron absorption from ferrous sulfate.

The Panel considers that the NF is a source from which iron is bioavailable.

## 3.9. Nutritional information

The NF is proposed by the applicant to be used as a source of iron, of which the NF contains 2–4%. The major fraction of the NF is protein (50–65%).



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Iron is an essential element that is required for oxygen transport, electron transfer, oxidase activities and energy metabolism (EFSA NDA Panel, 2015).

In 2015, the EFSA NDA Panel set dietary reference values for iron. The Population Reference Intake (PRI) for men and postmenopausal women was set at 11 mg/day. For premenopausal women, the Panel set a PRI of 16 mg/day. Furthermore, the Panel established PRIs of 11 mg/day for infants (7–11 months), 7 mg/day for children aged 1–6 years and 11 mg/day for children aged 7–11 years and boys aged 12–17 years. For girls aged 12–17 years, a PRI of 13 mg/day was set (EFSA NDA Panel, 2015).

EFSA performed an intake assessment of the anticipated daily intake of iron from the NF based on the applicant's proposed uses and maximum proposed use levels of iron provided by the NF (Section 3.7.4). The highest intakes (P95, in mg/day) of iron from the NF in the respective age groups are indicated in Table 8 below.

The background (dietary) intake of iron from food (not including food supplements) was previously estimated by the Panel based on the EFSA Comprehensive Database and the EFSA Nutrient Composition Database (EFSA NDA Panel, 2015). The estimated median and 95th percentile intakes from that Scientific Opinion are given in Table 8. For comparative reasons, a column with PRIs for iron as established by the Panel in 2015 is also included in the table.

**Table 8:** Intake of iron from the NF (at P95, when used as a food ingredient) and from the background diet (median and P95), in relation to the PRIs for iron

| Population group | Age (years) | Iron from the NF (mg/day)(1) | Range of median iron background intakes(2) (mg/day) | Range of iron background intakes at P95 (mg/day)(2) | PRI for iron(3) (mg/day) |
|---|---|---|---|---|---|
| Infants | < 1 | 8.7 | m: 1.9–5.9<br>f: 2.5–5.7 | m: 5.7–9.5<br>f: 5.7–9.0 | 11 |
| Young children | 1 to < 3 | 6.5 | m: 5.2–6.5<br>f: 5.0–6.4 | m: 7.9–11.4<br>f: 7.6–10.6 | 7 |
| Other children | 3 to < 10 | 3.4 | m: 7.7–11.2<br>f: 7.3–10.3 | m: 12.3–17.3<br>f: 11.0–16.3 | ≤ 6 years: 7<br>7–11 years: 11 |
| Adolescents | 10 to < 18 | 4.0 | m: 10.8–12.8<br>f: 8.9–15.3 | m: 17.6–22.2<br>f: 13.6–17.3 | 12–17 years (m): 11<br>12–17 year (f): 13 |
| Adults(4) | ≥ 18 | 2.8 | m: 12.2–14.3<br>f: 9.9–15.2 | m: 18.3–24.5<br>f: 14.1–34.9* | m: 11<br>f - premenopausal: 16(5)<br>f - postmenopausal: 11 |

NF: novel food; m: males, f: females, PRI: Population Reference Intake.
*: Survey of pregnant women in Latvia, P95 intakes in other countries/surveys are in the range of 15–18 mg/day.
(1): Does not include food supplements.
(2): EFSA NDA Panel (2015). Does not include food supplements.
(3): EFSA NDA Panel (2015).
(4): Includes all adult population groups (including pregnant and lactating women).
(5): Also covers needs for pregnant and lactating women.

The Panel notes that consumption of the NF would substantially contribute to iron intake, which, in combination with iron from the background diet, would be higher than the PRIs for iron for some population groups. The Panel notes that according to the intakes estimated in 2015, the iron intake is also above the PRIs in population groups with high background dietary iron intake.

No Tolerable Upper Intake Level (UL) has been set for iron by the SCF or EFSA (2004). Adverse gastrointestinal effects have been reported after short-term ingestion of non-haem iron preparations at doses of 50–60 mg/day, particularly if taken without food. However, EFSA considered that these adverse gastrointestinal effects are not a suitable basis to establish a UL for iron from all sources. The absence of convincing evidence of a causal relationship between iron intake or stores and chronic diseases was also noted (EFSA, 2004). The Panel notes that EFSA is currently assessing the setting of an UL for iron.

The Panel considers that taking into account the composition of the NF and the proposed conditions of use, the consumption of the NF is not nutritionally disadvantageous.



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### 3.10. Toxicological information

The applicant provided one acute oral toxicity study with the NF in Wistar rats, which were administered up to 2,000 mg NF/kg body weight (Suman, 2019, unpublished). The Panel considers that, in general, acute toxicity studies are of limited relevance for the safety assessment of novel foods.

No further toxicity studies were submitted by the applicant, who argued that the NF consists primarily of iron, phosphate and casein, which are normal constituents of the human diet.

The Panel considers that, in line with the Guidance on risk assessment of nanomaterials to be applied in the food and feed chain (EFSA Scientific Committee, 2021), the applicant presented sufficient evidence that the iron from the NF (in the form of soft nano-sized entities) does not bypass the homeostatic control of iron as a nutrient. Thus, the possible presence of the NF as soft nano-sized entities does not raise safety concerns as far as particle-related issues are concerned.

For these reasons, the Panel considers that no further toxicity studies are needed.

### 3.11. Allergenicity

The NF may contain up to 65% protein, owing to the sodium caseinate, which is one of the starting materials in the manufacturing process.

'Milk and products thereof' are listed under Annex II (i.e. substances or products causing allergies or intolerances) of Regulation (EU) No 1169/2011[7].

The Panel considers that the intake of the NF can cause allergic reactions similar to those arising from consuming milk and dairy products.

## 4. Discussion

The NF is IMP, which is a complex of iron, casein and phosphate. The NF is proposed by the applicant to be used as a source of iron, of which the NF contains 2–4%. The information provided on the composition is sufficient for characterising the NF.

The production process is sufficiently described and does not raise safety concerns.

The applicant intends to market the NF as an ingredient in a number of food categories, in food supplements, in total diet replacement for weight control and in FSMPs.

High intake estimates (at the 95th percentile) of iron from the NF (and not the NF itself) when used as a food ingredient, are 5.0 mg/day in adults, 4.6 mg/day in adolescents, 3.9 mg/day in 'other children', 6.5 mg/day in young children and 8.7 mg/day in infants in the European Union. The Panel notes that the consumption of the NF would substantially contribute to iron intake which, in combination with iron from the background diet, would be higher than the PRIs for some population groups. No UL has been set for iron by the SCF or EFSA, but EFSA is currently assessing the setting of an UL for iron. The Panel considers that, taking into account the composition of the NF and the proposed conditions of use, the consumption of the NF is not nutritionally disadvantageous.

The studies provided for ADME and bioavailability indicate that iron from the NF is bioavailable.

The Panel considers that overall the evidence indicates that upon ingestion the NF would undergo digestion into small peptides to yield iron-bound caseinophosphopeptides that are normal constituents of the human diet, and that the iron from the NF does not bypass the homeostatic control of iron as a nutrient. Therefore, no toxicity studies with the NF are required.

## 5. Conclusions

The Panel concludes that the NF, IMP, is safe under the proposed conditions of use.

The Panel also concludes that the NF is a source from which iron is bioavailable.

### 5.1. Data claimed as proprietary by the applicant

The Panel could not have reached the conclusions on the safety of the NF under the proposed conditions of use without the following data/studies that were claimed as proprietary by the applicant: certificates of analyses, *in vitro* digestibility report ('Annex G'), *in vitro* study (Sabatier et al., 2020), human study (Henare et al., 2019).



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## 6. Steps taken by EFSA

1) On 09/10/2020 EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of iron milk proteinate Ref. Ares(2020)5381834.
2) On 09/10/2020, a valid application on iron milk proteinate, which was submitted by the company Société des Produits Nestlé S.A, was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2020/1866) and the scientific evaluation procedure was initiated.
3) On 17/03/2021, 23/09/2021 and 28/01/2022 EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4) On 17/08/2021, 11/01/2022 and 22/07/2022 additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
5) During its meeting on 4 August 2022, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of iron milk proteinate as a NF pursuant to Regulation (EU) 2015/2283 and bioavailability of iron from this source in the context of Directive 2002/46/EC.

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## Abbreviations

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|---|---|
| ADME | absorption, distribution, metabolism and excretion |
| ANS | Panel on Food Additives and Nutrient Sources added to Food |
| AOAC | Association of Official Analytical Associations |
| APHA | American Public Health Association |
| bw | body weight |
| CFU | colony forming units |
| DHA | docosahexaenoic acid |
| DLS | dynamic light scattering |
| FePP | ferric pyrophosphate |
| FSMPs | Foods for Special Medical Purposes |
| GMP | Good Manufacturing Practice |
| HACCP | Hazard Analysis Critical Control Points |
| ICC | iron-casein complex |
| ICP-MS | inductively coupled plasma mass spectrometry |
| ICP-OES | inductively coupled plasma-optical emission spectroscopy |
| IMP | iron milk proteinate |
| LC–MS/MS | liquid chromatography coupled to tandem mass spectrometry |
| MPN | most probable number |
| NDA | Panel on Nutrition, Novel Foods and Food Allergens |
| NF | novel food |
| NMR | nuclear magnetic resonance |
| NRV | Nutrient Reference Value |
| P | phosphorus |
| PCR | polymerase chain reaction |
| PRI | Population Reference Intake |
| RH | relative humidity |
| RLP | Recognized Laboratory Programme |
| RTD | ready-to-drink |
| RTE | ready-to-eat |
| SCF | Scientific Committee on Food |
| SDS-PAGE | sodium dodecyl sulfate–polyacrylamide gel electrophoresis |
| SEC | size exclusion chromatography |
| TEM-EDS | transmission electron microscopy - energy dispersive X-ray scattering |
| UL | Tolerable Upper Intake Level |
| w/v | weight per volume |



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