Safety Assessment of Vitalarmor® GF-100 Whey Protein Isolate

Name of Applicant: Armor Protéines S.A.S., Saint Brice en Coglès, France

Contact person(s): Mr Emmanuel Treuil

Novel Food Classification: *Article 1.2(e)*

Introduction

An application for the authorisation of a basic whey protein isolate (Vitalarmor® GF-100) was submitted to the Food Safety Authority of Ireland (FSAI) by Armor Protéines S.A.S. of France in accordance with *Article 4* of the novel food Regulation (EC) No. 258/97. The application was accepted by the FSAI on August 22nd, 2016.

Vitalarmor® GF-100 is produced from raw cow's milk by physical separation and concentration techniques which are optimised to specifically isolate the minor basic whey proteins (primarily lactoferrin, and lactoperoxidase). The production process is broadly similar to that used to produce existing milk-derived products such as whey protein isolate and whey protein concentrate. The novel ingredient is made up of ≥ 90% protein, and is intended for use in infant and follow-on formulae, dietary foods for weight control (specifically meal replacement beverages), dietary foods for special medical purposes and food supplements. In accordance with the allergen labelling requirements set out in Regulation (EU) No 1169/2011, any food with added Vitalarmor® GF-100 will be labelled as containing 'milk'. The applicant concludes that while lactoferrin is an authorised novel food within the EU, no foods with added lactoperoxidase have been identified.

The novel ingredient falls under *Article 1.2(e)* of the novel food Regulation (EC) No 258/97: *"foods and food ingredients consisting of or isolated from plants and food ingredients isolated from animals, except for foods and food ingredients obtained by traditional propagating and breeding practices and which have a history of safe food use*". In order to assess wholesomeness, the application dossier was prepared pursuant to Class 2 of Commission Recommendation 97/618/EC; *"Complex NF from non-GM source",* and subclass (1); *"the source of the NF has a history of food use in the Community".*

I. Specification of the novel food

The novel ingredient is a yellow-grey powder consisting primarily of protein (≥90%) moisture (≤6%), fat (≤4.5%), ash (≤3.5%) and lactose (≤3.0%). The fractionation process used to produce the novel ingredient removes acidic proteins including casein and the two major whey proteins β-lactoglobulin and α-lactalbumin, thereby isolating the minor basic whey proteins lactoferrin and lactoperoxidase.

The proteins in Vitalarmor® GF-100 are primarily lactoferrin (47%) and lactoperoxidase (26%), with the remainder (approximately 20%) made up of varying low levels of other milk proteins including polymeric immunoglobulin receptor (secretory component), complement C3, β-lactoglobulin, α-S1-casein, the bioactive proteins TGF-β2 and IGF-1, and glycosylation-dependent cell adhesion molecule 1 (lactophorin). The applicant notes that the proteins may be partially denatured in the final product following pasteurisation.

Relative Content	Protein Constituents in Vitalarmor® GF-100
1	Lactoferrin (47%)
2	Lactoperoxidase (26%)
3	Polymeric immunoglobulin receptor (secretory component)
4	Complement C3
5	β-lactoglobulin
6	α-S1-Casein
7	Glycosylation-dependent cell adhesion molecule 1 (lactophorin)
8	Pancreatic ribonuclease
9	Tetranectin (CLEC3B: C-type lectin domain family 3 member B)
10	β-2-glycoprotein 1 (apolipoprotein H)

The data on bulk stability studies of the novel ingredient indicate that it is stable for up to 45 months when stored at 20°C at a relative humidity of 40-50%. However, an increase in

moisture level (up to 8%) is apparent in some batches following storage for up to 27 months and longer, which means that the moisture content specification is not fully reliable. There are no studies provided on the stability of the novel ingredient in the different food products and supplements as proposed, while the effects on stability caused by further processing has not been established. However, similar milk-derived proteins have already been used for some time in various food products and therefore any processing-related effects should be comparable.

II. Effect of the production process applied to the novel food

Vitalarmor® GF-100 is manufactured in accordance with current good manufacturing practice (cGMP) and in line with HACCP principles. The production plant producing the novel ingredient is certified to ISO 22000 standard. The production process starts with raw cow's milk from which the cream is separated. Basic whey proteins are then isolated from the skimmed milk by a series of physical separation and purification steps. Separation of the minor whey protein isolate is achieved through ion-exchange chromatography, a common technique used within the dairy industry. Sodium chloride (NaCl) is used to elute the basic whey proteins from the resin which is then concentrated by ultrafiltration before being pasteurised, spray dried and packaged. The isolation and purification process is described in detail and summarised in a process flow which outlines the critical control points.

III. History of the organism used for the novel food

The novel ingredient is isolated from cow's milk, a normal component of the human diet with an established history of safe use, except of course for people with an allergy to milk or lactose intolerance.

IX. Anticipated intake/extent of use of the novel food

The data provided in this application regarding anticipated intake and extent of use is complex due to the number and different type of foods and supplements to which the novel ingredient is to be added. In addition, a number of different datasets are utilised to estimate intakes while consideration is also given to the intakes of the primary constituents, lactoferrin and lactoperoxidase.

The applicant intends to add the novel ingredient to infant and follow-on formulae, dietary foods for weight control diets, dietary foods for special medical purposes and food supplements. The proposed maximum levels are summarised in Table 1 and the use levels for

younger populations (≤3 years) will be adjusted so that the levels of total TGF-β_2 (an indicator protein) in foods/formulae for younger children are comparable to those in mature human milk. The level of 300 mg/kg of Vitalarmor® GF-100 added to infant and follow-on formula represents a worst-case scenario as protein denaturation may occur naturally over time and/or as a result of processing.

Table 1: Proposed maximum Use Levels for Vitalarmor® GF-100

Food Category	Suggested Serving Size	Max. Level per Serving	Proposed Max. Use Level
Infant formula	N/A	300 mg/kg (powder) equivalent to 3.9 mg/100 ml (reconstituted)	0.03% (powder) 0.0039% (reconstituted)
Follow-on formulae	N/A	300 mg/kg (powder) equivalent to 4.2 mg/100 ml (reconstituted)	0.03% (powder) 0.0042% (reconstituted)
Dietary foods for special medical purposes (Medical Foods)	Case-by case	Up to 610 mg/day	Up to 610 mg/day
Dietary foods for special medical purposes (Meal replacement beverages)	250 g	100 mg/250 g meal replacement	0.04%
Food supplements in any form	N/A	Up to 610 mg per recommended daily dose	Up to 610 mg

The following datasets were utilised for intake estimations: 1) EFSA's comprehensive database, ii) the 2011 UK's Diet and Nutrition survey of Infants and Young Children (DNSIYC) aged 4-17 months and the UK National Diet and Nutrition Survey (2008-2012) of individuals aged 18 months and over. In addition, to assess intakes of formula by younger children (<6 months), intake estimates originating from the DONALD study (the German Dortmund Nutritional and Anthropometric Longitudinally Designed study) were used. The UK NDNS data covers individuals aged 18 months and older while the UK DNSIYC covers infants and young children aged between 4 and 17 months. However, the UK DNSIYC does not address 0 to 4-month-old infants and so potential intakes of Vitalarmor® GF-100 from infant and follow-on formulae in this group were estimated using the DONALD study.

The applicant states that the estimation of meal replacement intakes was difficult due to the low numbers of people consuming them. However, the intake (worst case scenario) of the novel ingredient by adults via meal replacement beverages was estimated to be 300 mg/day or 4.29 mg/kg body weight/day (for a 70 kg adult). The applicant also estimates intakes by

adults from other foods for special medical purposes, albeit specific levels of use in these foods would be adjusted on a case-by-case basis so that total intake of Vitalarmor® GF-100 would not exceed 610 mg/day. In this scenario, for a 70 kg adult intakes would not exceed 8.7 mg/kg body weight/day.

The maximum recommended intake of Vitalarmor® GF-100 via food supplements for adults is 610 mg per day, reduced to ≤58 mg/day for children less than three years of age.

Based on rat studies, the applicant proposes a NOAEL for Vitalarmor® GF-100 of 2,000 mg/kg bw/day (the highest dose tested), and 600 mg/kg bw/day for foods intended for infant consumption. The applicant did not provide an Acceptable Daily Intake level on the basis that cow's milk is part of the normal human diet for all age groups, though it is unclear if an uncertainty factor for inter-species differences has been applied. NOAEL values were provided for compositionally related milk protein fractions containing a combination of lactoferrin, lactoperoxidase and bovine milk basic protein. These ranged from 600-3000 mg/kg bw/day and were invariably the highest dose tested.

Commission Implementing Decision 2012/725/EU authorised the use of bovine lactoferrin as a novel ingredient in a range of food categories, including infant and follow-on formula and foods for special medical purposes, among others. The proposed use levels of Vitalarmor® GF-100 in the specified food categories would not result in the levels of lactoferrin exceeding those set out in Commission Implementing Decision 2012/725/EU. The applicant advises that Vitalarmor® GF-100 food supplements are not intended for those already taking meal replacement beverages containing the novel ingredient. Labelling requirements for both product categories should be sufficient to help avoid over-consumption.

X. Information from previous human exposure to the novel food or its source

Exposure to the proteins in Vitalarmor® GF-100 occurs routinely through the consumption of cow's milk and dairy products derived from cow's milk. Vitalarmor® GF-100 is presently not used as a food ingredient in the EU, though similar cow's milk derived ingredients such as whey protein isolates, whey protein concentrates and demineralised whey powder are already in use. Vitalarmor® GF-100 achieved GRAS status in 2015 in the USA for use as an ingredient in infant and toddler formulas, meal replacement beverages and medical foods (food supplements not included).

While the addition of lactoperoxidase as a food ingredient is not recorded in the EU at present, it is available as an ingredient in dietary supplements in the US (1-2mg/serving), as a

processing aid in New Zealand and as a food additive in Japan. Bovine lactoferrin has GRAS status in the US and is marketed in supplements at doses up to 1,000mg/day. Commission Implementing Decision 2012/725/EU authorised the use of bovine lactoferrin as a novel ingredient in a range of food categories. At the proposed use levels, the lactoferrin in Vitalarmor® GF-100 would be within the maximum use levels in infant and follow-on formula as well as foods for special medical purposes as set out in Annex II of Commission Implementing Decision 2012/727/EU.

XI. Nutritional information on the novel food

The novel ingredient contains lactoferrin (an iron-binding protein) which is one of the main whey proteins present in cow's milk and human breast milk and also found in various secretions. It also contains lactoperoxidase (a haem-containing enzyme with peroxidase activity) found in human colostrum and other bodily secretions, where it may have anti-bacterial properties. Additional proteins are also present in Vitalarmor® GF-100 but do not contribute significantly to its nutritional value. Addition of the novel ingredient to a food means an additional source of iron in that food, although the specifications of the novel ingredient limit the level of iron in the final product to ≤25 mg iron/100g. Intake estimates show that iron uptakes could reach up to 10 µg/day in infants up to 4 months of age, and 150 µg iron/day adults. These intake levels are below those considered by EFSA to be adequate for infants and toddlers and below the daily reference intake for adults. The applicant states that, for use in infant and follow-on formulae, the iron content of the novel ingredient containing infant formula will have to meet the compositional standards of infant formulae and follow-on formulae established in Directive 2006/141/EC.

There are no studies available on the metabolic fate of Vitalarmor® GF-100, though the applicant does provide information on the metabolism of the major protein fractions (lactoferrin and lactoperoxidase). The applicant provides evidence that lactoferrin ingested by humans, particularly infants, is resistant to digestion in the upper gastrointestinal tract, passing through the stomach largely intact. Under worst case scenario estimates, i.e. maximal combined intakes of foods/supplements containing Vitalarmor® GF-100 at maximal limits for iron (25 mg/100g), consumption of formula, medical food or food supplements containing the novel ingredient would yield an additional 14.5 – 20µg/day of iron to the diets of 0-4 month old infants and 230 µg/day to adults. This is within the levels deemed adequate for infants

(0.3–8 mg for infants and young children) and the daily reference intake for adults (14 mg/day).

XII. Microbiological information on the novel food

The raw material (milk) is sourced in France and is therefore subject to EU legislation concerning the production of milk and dairy products. This includes the monitoring for bovine tuberculosis, but not for *Brucella abortus* as France has been officially free of bovine brucellosis since 2012. The potential for microbiological contamination of the finished product is controlled by ensuring the microbiological quality of the starting material. Furthermore, the production process and down-stream processing of the novel ingredient includes temperature control and pasteurisation, limiting the potential for microbial contamination. Batch analysis of Vitalarmor®GF-100 indicates the absence of yeasts and moulds, *Escherichia coli, Staphylococci, Salmonella, Listeria and Cronabacter spp.*

XIII. Toxicological information on the novel food

Metabolic fate

Studies concerning the metabolic fate of Vitalarmor® GF-100 are not available. However, the primary proteins, lactoferrin and lactoperoxidase are naturally present in cow's milk and so their metabolic fate should be similar. Lactoferrin ingested by humans, particularly infants, is resistant to digestion in the upper gastrointestinal tract, passing through the stomach largely intact. Following absorption, it is distributed to a number of organs including the liver, kidney, gall bladder, brain and spleen. Unabsorbed lactoferrin is excreted in the faeces, with some urinary excretion also reported. There is less information available regarding the metabolic fate of lactoperoxidase.

Toxicological Studies

The toxicity of the novel ingredient was investigated in a sub chronic oral toxicity study in rats where administration did not result in any treatment-related clinical signs of toxicity. The NOAEL for Vitalarmor® GF-100 was established as 2,000 mg/kg body weight/day, the highest dose tested. The toxicity was also investigated in a 6-week juvenile rat oral toxicity study, there were no treatment-related mortalities or clinical signs of toxicity and a NOAEL of 600 mg/kg bw/day established for foods intended for infants (the only dose tested). In addition, the novel ingredient did not show any mutagenic potential using the Ames test. Toxicology studies on bovine lactoferrin have previously been evaluated by EFSA in which

they concluded that the NOAEL was 2,000 mg/kg body weight/day. Toxicology studies on protein fractions similar to the novel ingredient were also carried out by the applicant with similar positive outcomes. Vitalarmor® GF-100 did not show a genotoxic potential.

Allergenicity

The applicant confirms that foods containing Vitalarmor® GF-100 will be labelled as containing "milk", in line with the allergen labelling requirements set out in Regulation (EU) No 1169/2011.

Conclusions

This application seeks to authorise a milk protein fraction (Vitalarmor® GF-100) in infant and follow-on formulae, food supplements, meal replacement beverages and dietary foods for special medical purposes. The primary proteins in this 90% milk-protein product are lactoferrin and lactoperoxidase. Lactoferrin has already been assessed and authorised as a novel food ingredient in the EU, though lactoperoxidase as an individual ingredient has not. The level of lactoferrin exposure through the proposed consumption of Vitalarmor® GF-100 should not exceed the intake values for the authorised lactoferrin. The safety of Vitalarmor® GF-100 is supported by toxicological studies on the novel ingredient itself and related milk-derived ingredients. Though the assessments were conservative and incorporate worst-case scenarios, there does not appear to be any adverse effects on the nutritional status of individuals consuming Vitalarmor® GF-100. The applicant advises that food supplements containing Vitalarmor® GF-100 are not intended for those already taking Vitalarmor® GF-100 meal replacement beverages and so contemporary labelling requirements should be sufficient to help prevent against over-consumption.

Recommendation

The Food Safety Authority of Ireland has not identified any safety concerns associated with the consumption of Vitalarmor® GF-100 at the proposed use levels in infant and follow-on formulae, food supplements, meal replacement beverages and dietary foods for special medical purposes. The FSAI is therefore of the opinion that Vitalarmor® GF-100 meets the criteria for novel food set out in *Article 3.1.* of the novel food Regulation (EC) No 258/97.