AGENCE FRANÇAISE
DE SÉCURITÉ SANITAIRE
DES ALIMENTS

AFSSA Request No 2010-SA-0112

Maisons-Alfort, 3 June 2010

THE DIRECTOR GENERAL

OPINION

of the French Food Safety Agency on the request for an assessment of the initial assessment report by the Dutch authorities concerning the placing on the market of a novel food ingredient: bovine lactoferrin

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The French Food Safety Agency (AFSSA) received a solicited request on 16 April 2010 from the Directorate General for Competition, Consumer Affairs and Fraud Control for an assessment of the initial assessment report by the Dutch authorities concerning the placing on the market of a novel food ingredient: bovine lactoferrin.

1. BACKGROUND

An application for the placing on the market of bovine lactoferrin was submitted to the Dutch authorities under Regulation (EC) No 258/97 concerning novel foods and novel food ingredients. The applicant wishes to incorporate purified bovine lactoferrin obtained from cow's milk into a range of products (dairy products, foodstuffs intended for specific populations, etc.) as an ingredient. The initial assessment report drawn up by the Dutch authorities has been submitted to AFSSA for any comments or objections.

According to the applicant, the novel ingredient (NI) can be regarded as belonging to category (e) as defined in Article 1(2) of the aforesaid Regulation, which includes 'foods and food ingredients consisting of or isolated from plants and food ingredients isolated from animals […]'.
The applicant considers that the NI belongs to class 2.1 as defined in Recommendation 97/618/EC of the European Commission, which includes complex novel foods and novel food ingredients from non-GM sources.

The Dutch committee notes that the NI *could also belong to class 1.1, which includes pure chemicals or simple mixtures from non-GM sources. This is of no consequence, however, since the same assessment procedure applies in both cases.*

This classification does not give rise to any comment from AFSSA. AFSSA notes that lactoferrin is authorised in food supplements within the European Union. GRAS status was granted to bovine lactoferrin in 2001 by the FDA. In 2008, AFSSA issued an Opinion concerning the assessment of the initial report by the Belgian authorities on the placing on the market of bovine lactoferrin on the basis of substantial equivalence with the lactoferrin present in cow's milk. The European assessment procedure relating to this novel ingredient has not yet been completed.

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2. METHOD OF EXPERT APPRAISAL

The collective expert appraisal was carried out by the Expert Committees (CESs) on Human nutrition and Additives, flavourings and processing aids, which met on 6 May 2010 and 20 May 2010 respectively.

3. DISCUSSION

AFSSA's line of reasoning is based on the opinions of the Expert Committees (CESs) on 'Human nutrition' and 'Additives, flavourings and processing aids', the main points of which are presented below:

3.1. Specification of the NI

Regarding the composition of the NI:
Bovine lactoferrin is a protein consisting of a single glycosylated polypeptide chain containing disulphide bonds. Its amino acid sequence is known. It is a transferrin with a bilobed polypeptide chain. Each lobe is a site for iron binding, which allows two iron molecules to be bound per molecule of protein. The applicant estimates the content of the end product to be 120 mg/kg of NI.
The applicant states that the NI contains a minimum of 93% protein, of which over 95% is lactoferrin. Other milk proteins are likely to be present in the product in small quantities, in particular casein, α-lactoglobulin and β-lactoglobulin. The product contains no more than 4.5% water and 1% ash. The total heavy metals content (cadmium, lead, arsenic, mercury, copper) does not exceed 1 mg/kg of the NI. On the basis of stability tests carried out for up to six years, the applicant states that the chemical characteristics (protein, dry matter and mineral content, pH, iron binding capacity) and microbiological characteristics (bacterial count) of the product are stable.
The applicant has supplied the results of six production batch analyses.

On the basis of these analyses, the Dutch committee considers that the composition of the NI is constant in the different batches and that it complies with the values specified by the applicant. At the request of the Dutch committee, the applicant provided data relating to the iron content of different production batches. For three batches from the production period 2006-2008, the iron content was between 100 and 216 mg/kg; for 11 batches from 2009, the iron content was around 140 mg/kg. The applicant agreed to specify a maximum iron content, which has been set at 250 mg/kg.

The Dutch committee notes that the applicant has not provided data relating to heavy metals content in the batches tested. On the basis of a comparison of maximum contents specified by the applicant with the maximum permissible contents set for the heavy metals by the Netherlands Institute for Public Health (Baars et al., 2001), the Dutch Committee considers that the higher limits specified for cadmium (<0.02 mg/kg), lead (0.2 mg/kg), arsenic (0.2 mg/kg), mercury (<0.01 mg/kg) and copper (1.5 mg/kg) are sufficiently low.

In conclusion, the Dutch committee considers that the information available on the NI makes it possible to assess its composition. It does not contain any contaminants likely to have adverse effects. The Dutch committee considers that the stability of the NI is guaranteed in the long term.

In agreement with the Dutch committee, AFSSA considers that the information relating to the specification of the NI makes it possible to characterise the latter satisfactorily. The stability of the product is guaranteed at room temperature.

Regarding the stability of the NI during its production process or that of the products into which it is likely to be incorporated:

The thermal treatment of bovine lactoferrin can modify the native state (molecular structure in space) of the protein and its related properties. The applicant states that the degree of denaturation depends on many parameters, including the intensity of thermal treatment, pH and the level of iron saturation. The applicant presents the results of studies testing the stability of the NI during its incorporation into certain products (dairy products, infant formula and follow-on formula etc.). The applicant stresses that the thermal treatments that are used do not denature the bovine lactoferrin.

The Dutch committee considers that there is nothing to substantiate this claim. Furthermore, it considers that denatured lactoferrin has, at most, the same activity as native lactoferrin. Thus when assessing the safety of the NI, the Dutch committee has regarded all lactoferrin as being in its native form.

AFSSA notes that the applicant does not justify the claim that the production process used does not denature bovine lactoferrin. A recent study on this subject (Schwarcz et al., 2008) shows in particular that the pasteurisation of milk is likely to bring about changes to the tertiary structure of bovine lactoferrin, and thus to the properties linked thereto. AFSSA would query the nutritional equivalence of native bovine lactoferrin and lactoferrin which has been more or less denatured.

3.2. Effect of the production process applied to the NI

The applicant describes the production process which enables bovine lactoferrin to be isolated from milk or products derived from cow's milk such as whey. The applicant states that the quality of the milk used for producing the NI is identical to that of milk intended for human consumption. Skimmed pasteurised milk or whey is obtained at the end of the first stage of the production process, and undergoes microfiltration in order to reduce the microbial load and the fat content. Ion-exchange chromatography followed by elution and filtration make it possible to isolate the protein. The product thus obtained is dried, sieved and packaged. The facilities are ISO 9000:2000 certified and the production processes are subject to HACCP (Hazard Analysis Critical Control Point) plans. In line with Regulation (EC) 853/2004 laying down specific hygiene rules for food of animal origin, the facilities have been approved for the production of dairy products.

The Dutch committee notes that the NI is produced according to techniques commonly used in the dairy industry. Although the applicant's dossier does not include any data relating to quality systems, the Dutch committee considers that there is no reason to doubt the safety of the production process.

In agreement with the Dutch committee, AFSSA considers that the production process of the NI does not pose any health problems and that it complies with European hygiene rules.

3.3. History of the organism used as the source of the NI

The NI is obtained from cow's milk or cow's milk derived products such as whey. The cow's milk used is a source which has been fully characterised.

The Dutch committee considers that the information provided by the applicant on the source of the NI is satisfactory.

This point does not elicit any special comments from AFSSA

3.4. Anticipated intake/extent of use of the NI

The applicant intends to incorporate the NI into a large range of product categories, at the maximum concentrations shown in Table 1 below:

Product categories	Maximum concentration of bovine lactoferrin (mg/100g)
Infant nutrition – infant formulas and follow-on formulas	100
Infant nutrition – growing-up milk	200
Infant nutrition – weaning foods	667
DFSMP* – liquid and powder formulas	125
DFSMP – enteral and parenteral nutrition	50
DFSMP – others	800
Sports nutrition – liquid and powder formulas	303
Sports nutrition – energy bars	4000
Non-alcoholic beverages	120
Cakes and pastries	1000
Cheese products	2000
Milk and dairy products	200
Snacks	1200
Confectionery	750

Table 1: Categories of products and maximum levels of enrichment intended by the applicant
* DFSMP: Dietary Foods for Special Medical Purposes

In order to estimate intakes of bovine lactoferrin, the applicant assumed that the products in the categories listed in Table 1 above were enriched to the maximum intended concentration. A detailed list of the products in each category into which the applicant intends to incorporate the NI is included in the applicant's dossier (Appendix A, Annex A1).

Regarding the estimates of intake of the NI for the general population:
Estimates of lactoferrin intake via the consumption of products in which the applicant intends its use are based on the individual consumption data collected in the third Dutch National Food Consumption Survey (DNFCS), carried out in 1997-1998. This survey did not assess the food consumption of children under one year of age, and so this population will be dealt with separately.
The average intake of lactoferrin was estimated for all individuals in the sample, including non-consumers (the results obtained when only consumers were included proved to be similar).

Assuming that the NI is incorporated at the maximum level into the carrier substances intended by the applicant, the levels of exposure which would be reached are shown in Table 2 below:

	Average (mg/d) Average (mg/kg bw*/d)	P 95 (mg/d) P 95 (mg/kg bw/d)	P 97.5 (mg/d) P 97.5 (mg/kg bw/d)
Total population	1531.4 **31.7**	3035.7 **130.9**	3568 **135.1**
Children 1-3 years	1670.6 **124**	2709.4 **202.7**	2898.3 **224.7**
Boys 4-9 years	1876.6 **80.4**	3111.3 **138.8**	3567.1 **165.4**
Girls 4-9 years	1723 **75.6**	2756.3 **125.0**	3012.8 **152.2**
Boys 10-18 years	2330.8 **45.8**	4202 **88.3**	4872 **123.3**
Girls 10-18 years	2000.2 **40.8**	3381.8 **75.0**	3771.4 **93.1**
Men over 19 years	1423.1 **17.9**	2930.4 **37.3**	3568 **42.9**
Women over 19 years	1378.6 **20.1**	2796.6 **41.4**	3297 **48.4**
Pregnant women	1845.4 **25**		

Table 2: Average exposure (95th and 97.5th percentiles) to the NI of the Dutch population according to the DNFCS 3. The sample of pregnant women only consisted of 50 individuals, which meant that it was impossible to calculate the exposure of higher percentiles for this population.
* bw: body weight

The average intake for the total population is 1.5 g/d. It reaches 2.0 and 2.3 g/d respectively for girls and boys of 10-18 years. The 97.5th percentile consumption reaches 4.8 g/d in these latter.

With regard to intake per kg of body weight (kg bw), the average intake is estimated to be 31.7 mg/kg bw/d. It reaches 124 mg/kg bw/d in children from 1 to 3 years. The 97.5th percentile consumption reaches 225 mg/kg bw/d in these latter.

The respective contributions of dairy products, non-alcoholic beverages, cakes and pastries to the intake of lactoferrin are 48%, 22% and 15% respectively, resulting in a cumulative contribution of 84%.

Regarding the estimates of intake of the NI for infants (0-1 years):
For infants between 0 and 6 months, the applicant estimated a consumption of infant formula of around 1.2 litres per day (on the basis of paediatric nutrition recommendations), which corresponds to a lactoferrin intake of 1.2 g per day (the applicant having estimated the concentration of lactoferrin in infant formula at around 100 mg per litre).
For infants over 6 months, the applicant referred to the results of a food consumption survey carried out over two days with 941 Dutch children. Only the results relating to infants between 8 and 10 months were used, which showed an average consumption of 608 g of milk and dairy products per day and of 189 g/d of weaning food (baby food, etc.). The applicant inferred an average intake of bovine lactoferrin of 1868 mg/d from these figures.
On the basis of a weight of 5.8 kg and 9.7 kg respectively for infants of 5 and 11 months (EFSA, 2008b), the applicant considers that intake of lactoferrin increases from 207 to 214 mg/kg of body weight/d during the first year of life.

The Dutch committee stresses that the applicant has carried out a satisfactory study into estimates of lactoferrin intake. It considers that the estimates obtained are most probably higher than the actual intakes which will be observed, taking into account the hypothesis used, whereby maximum levels would be incorporated. However, it is possible that the uses of lactoferrin or consumption of the intended carrier substances could be extended or increase over time. As the consumption data used date from 1997-1998, the Dutch committee has reservations as to whether it is relevant to extrapolate the results obtained to the current Dutch population.

Furthermore, the Dutch committee considers that the applicant does not specify the extent to which the consumption data used are representative of consumption in other European countries. It highlights in particular the significant differences in the consumption of dairy products between the different Member States (cf. Table 2.1 of Annex A to the applicant's dossier, EFSA 2008).

Furthermore, the Dutch committee points out that an estimate of exposure to bovine lactoferrin via the consumption of food supplements would be highly relevant for assessing the safety of its use. In response to a request submitted by the Dutch committee, and on the basis of sales data for a food supplement containing bovine lactoferrin marketed by the applicant, the latter has estimated the daily intake of lactoferrin in adolescents using the food supplement for skin problems to be 160 mg, or in other words 3-4 mg/kg bw/d, which is a relatively small contribution to the total intake (average intake from 10 to 18 years via the NI: 40-45 mg/kg bw/d). However, the Dutch committee notes that the current level of use of food supplements based on bovine lactoferrin from various manufacturers is unknown.

AFSSA notes the very high number of products likely to be enriched. Nearly 500 are listed in Annex A to the applicant's dossier.

AFSSA confirms the significant variations in food consumption between the different European countries. In France, significant differences have been recorded in particular for dairy products and non-alcoholic beverages[1]. The average consumption data for different categories of food in European countries (EFSA, 2008) show that consumption of dairy products is much higher in Finland, Iceland and Norway (437, 442 and 522 g/d respectively) than in the Netherlands (388 g/d). Consumption of non-

[1] The average consumption of milk and dairy products in France is estimated to be 206 g/d (AFSSA 2009), compared to 388 g/d in the Netherlands (EFSA, 2008). The consumption of non-alcoholic beverages is estimated to be 158 g/d in France, compared to 254 g/d in the Netherlands.

alcoholic beverages is also much higher in Belgium (346 g/d), Norway (416 g/d) and Iceland (426 g/d) than in the Netherlands (254 g/d). Estimates of lactoferrin intake for the populations of certain countries are thus likely to be higher than the intakes estimated by the applicant for the Netherlands.

3.5. Information from previous human exposure to the NI or its source

Current exposure

Bovine lactoferrin is naturally present in small quantities in cow's milk. The applicant estimates its concentration to be around 100 mg per litre. On the basis of consumption data for the Dutch population (aged over 1 year), the applicant estimates that the current average consumption of bovine lactoferrin via dairy products is 40 mg per day. In infants, the intake of lactoferrin via dairy products is estimated to be 63 mg/d. In the United States, the 95th percentile intake is estimated at 100 mg/d in adults.

The Dutch committee points out that the quantity of lactoferrin in cow's milk is likely to vary considerably. In this regard, it cites the study by Yamauchi et al. (2000), who reports concentrations of between 100 and 400 mg/L, as well as concentrations reported by the Australian authorities of between 20 and 200 mg/L.

The Dutch committee considers that almost all bovine lactoferrin is denatured during the thermal treatment of milk. It cites AFSSA's Opinion of 2 September 2008 (AFSSA, 2008), stating that the average intake of non-denatured bovine lactoferrin is estimated to be 5.3 mg/d, coming from unpasteurised milk and cheeses made from unpasteurised milk.

The Dutch committee notes that there are no consumption data in Europe relating to food supplements based on bovine lactoferrin. The applicant states that the consumption of some of them can result in an intake of up to 1200 mg of lactoferrin per day.

As stated in its previous Opinion (AFSSA, 2008), AFSSA considers that the average total intake of lactoferrin by an adult can vary from 20 to 50 mg/d, according to the type of dairy products consumed.

Human clinical studies relating to exposure to the NI:

The Dutch committee has examined 30 clinical studies using bovine lactoferrin presented by the applicant. It highlights that all these studies bar one use products based on lactoferrin originating from companies other than that of the applicant, but which can however be considered equivalent thereto. Furthermore, as pointed out by the applicant, the Dutch committee notes that the aim of these studies is to assess the efficacy of lactoferrin, and not how safe it is to use.

As far as infants are concerned, the Dutch committee considers that a single study (King et al., 2007) is relevant in terms of assessing how safe bovine lactoferrin is to use (the other studies only assess the product's efficacy). This is the only study using bovine lactoferrin manufactured by the applicant. It is a controlled double-blind trial; for one year, the infants in the experimental group received 850 mg of lactoferrin per litre of infant formula compared to 102 mg in the control group (n=26 infants/group). Similarly to the authors, the Dutch committee notes the small size of the study (n=26/group). However, taking into account the in-depth nature of the study otherwise, it considers that these data are sufficient and concludes that the long-term consumption of bovine lactoferrin at doses similar to the level of use intended by the applicant does not result in adverse effects on the growth or health of infants.

As regards children, a single study has been used by the Dutch committee to assess the safety of the product. It notes the study's small size (n=26 children/group at the start). The study relates to children between 1 and 3 years receiving 500 mg of bovine lactoferrin per day for 9 months (controlled double-blind study). The authors state that no adverse effect was observed.

Nineteen studies carried out on adults are presented by the applicant. Five of them relate to subjects in good health receiving up to 2 g of lactoferrin per day. The treatment was well tolerated by the participants.

The 14 other studies relate to patients often suffering from Helicobacter pylori infections or hepatitis C, sometimes on a chronic basis. The doses of lactoferrin received by the patients were up to 3.6 g/d in three studies and 7.2 g/d in one study (Okada et al., 2002). On the basis of this last study, the applicant concludes that there are no adverse health effects of bovine lactoferrin at doses up to 7.2 g/d in adults. The Dutch committee notes that the

patients participating in this study were suffering from chronic hepatitis C, and that such a dose has not been tested in individuals in good health. The committee stresses that this is the only study in which adverse effects were reported (in 3 of the 15 patients receiving 7.2 g/d of lactoferrin and one patient receiving 3.6 g/d). The adverse effects reported were as follows: skin rashes, loss of appetite, constipation, diarrhoea. These effects, which were reversible, were not accompanied by significant changes to the blood parameters measured. The Dutch committee notes the absence of serious adverse effects.

In conclusion, the Dutch committee considers that, generally speaking, no major adverse effect clearly linked to the daily consumption of bovine lactoferrin for several months appeared at the quantities to be used as proposed by the applicant.
Therefore on the basis of this data set, the use of bovine lactoferrin under the conditions specified by the applicant does not give rise to concern on the part of the Dutch committee. It notes, however, that there has been no large-scale safety study carried out with the product on subjects in good health.

AFSSA notes that the studies presented show no adverse effects following the ingestion of bovine lactoferrin in adults, children or infants, even for intakes greatly exceeding normal ingestion. It should be noted, however, that the aim of these studies was to assess the efficacy of the NI, and not its safety. AFSSA stresses the absence of any studies directly assessing the long-term safety of bovine lactoferrin for subjects in good health. Such studies would yield additional information in order to assess the safety of use of bovine lactoferrin.

3.6. Nutritional information on the NI

According to the applicant, enriching food with lactoferrin could enable this lactoferrin to join the endogenous lactoferrin synthesised by the body. The applicant states that 70% of the amino acid sequence is the same in human and bovine lactoferrin.

The Dutch committee considers that the products resulting from digestion of the two proteins are not strictly equivalent. The peptides formed are of different types and have different activities, resulting in particular from their transformation by microbial enzymes in the colon.

The Dutch committee compared the nutritional intake via bovine lactoferrin originating from the enrichment of products with the NI to the total protein intake in different age groups of the Dutch population. The ratio is highest in infants, in the case of which 5-10% of protein intake may come from bovine lactoferrin.

Enrichment with 1 g of lactoferrin per litre of infant formula or follow-on formula, as proposed by the applicant, corresponds to a maximum iron intake of 0.25 mg. The Dutch committee notes that this value corresponds to 2-3% of the maximum content as laid down by Directive 2006/241 on infant formulas and follow-on formulas. The study by King et al. (2007) in which infants received bovine lactoferrin at a dose of 850 mg per litre of infant formula or follow-on formula for one year showed no changes in haemoglobin or haematocrit levels in the blood.

In individuals aged over one year, the intake of iron bound to lactoferrin is in the order of 0.14 mg/g of the NI, but never exceeds 0.25 mg/g of the NI (see results of analysis of production batches). In the case of consumers for whom intakes are likely to be highest (4 g of the NI/d in the case of adult men at the 97.5th percentile), this could result in an intake of 1 mg of iron per day via the NI. Having compared this additional intake to the intake via normal food (between 6 and 13 mg per day according to age and sex), the Dutch committee concludes that there are no adverse effects linked to enrichment.

Digestion and absorption of the NI:
The applicant provides a long description of aspects relating to the digestion and absorption of lactoferrin, comparing the differences between bovine and human lactoferrin, the differences between the results obtained in humans and in animals and the differences observed between adults and children.

The Dutch committee has limited its analysis to methods of absorption and digestion of bovine lactoferrin in humans. It notes that the digestion of this protein depends on age, due to the gradual development of the intestinal function in humans. The proteolysis of bovine lactoferrin is relatively slow in newborns. Due to incomplete proteolysis, bovine lactoferrin

can be found in the faeces of infants. The Dutch committee notes the absence of similar studies carried out on adults.
The applicant states that non-degraded lactoferrin can be absorbed and enter the bloodstream in infants. The Dutch committee considers that this has only been shown for species-specific lactoferrin (via breast milk), and that the dossier does not contain any information showing absorption of bovine lactoferrin in infants. However, the Dutch committee notes that this has been shown in adult mice with mature intestinal function (Fischer, 2007).

In conclusion, the Dutch committee considers that there is no reason to suppose that the digestion of lactoferrin should differ from that of other dietary proteins. It appears, however, that bovine lactoferrin is relatively resistant to proteolytic enzymes in the intestinal tract. Finally, the Dutch committee notes the lack of information relating to the intestinal absorption of bovine lactoferrin in humans.

In agreement with the Dutch committee, AFSSA notes the lack of data relating to methods of digestion and absorption of bovine lactoferrin, in particular in adults. AFSSA confirms that the digestion of bovine lactoferrin can be deemed not to differ from that of other food proteins. The relative resistance of bovine lactoferrin to proteolysis could favour the absorption of more or less intact molecules, or even activity of this protein in the digestive tract.
Furthermore, the consequences of enrichment with the NI for protein and iron nutrition do not give rise to any concerns on the part of AFSSA.

AFSSA notes that the beneficial health effects of the NI claimed by the applicant are not the subject of the present assessment, and that these should be proven on a case-by-case basis for the intended carrier substances if the NI receives marketing authorisation.

3.7. Microbiological information on the NI

The applicant notes that the production process does not involve the use of microorganisms. Furthermore, the process is subject to an HACCP procedure in order to ensure its safety. The applicant has tested for the presence of several undesirable microorganisms (*Salmonella*, *Staphylococcus*, mould etc.) in six production batches. The quantities detected proved to be below the specification limits of the NI.

On the basis of this information, the Dutch committee considers there is no possibility of any microbiological risk.

In agreement with the Dutch committee, and taking into account the manufacturing and control processes, AFSSA considers that the NI is not likely to present any microbiological risk.

3.8. Toxicological information on the NI

The Dutch committee notes that almost all of the toxicology studies have been carried out with bovine lactoferrin from a Japanese manufacturer. The bovine lactoferrin content of the product thus used is at least as high as that of the NI. The Dutch committee notes that the composition of the other dairy proteins present is not specified and that the iron content is lower than that of the NI. However, it recognises that the product used is representative of the NI proposed by the applicant for the assessment of safety.

Genotoxicity study:
A test for bacteria was carried out in 2000 on four *Salmonella* strains (TA98, 100, 1535, 1537) and an *Escherichia coli* strain, up to a dose of 5000 µg per plate with and without metabolic activation including a pre-incubation stage. No mutagenic potential was observed.

In the opinion of the Dutch committee, this test is sufficient to rule out any mutagenicity resulting from the NI.

AFSSA shares the opinion of the Dutch experts. A test limited to bacteria is sufficient in the case of a protein.

Toxicity studies:

- Single-dose study:

The results of this study, carried out in 1997 on rats, show no toxicity up to 200 mg/kg of body weight.

- Four-week study:

This study was carried out in 1997 on adult Sprague-Dawley rats which were tube fed doses of bovine lactoferrin of 0, 200, 600 and 2000 mg/kg of body weight (bw)/day (d). No cases of death were reported. Some sporadic effects were reported which were not connected to the substance being tested (persistent hyaloid artery in the eye, pulmonary dilation, ulcers, stomach erosions, pulmonary haemorrhagic foci, etc.), and which affected only one or two animals per group (including controls). According to this study, the NOAEL[1] is 2000 mg/kg bw/d.

- Thirteen-week study:

This study (Yamauchi et al., 2000) was carried out on adult Sprague-Dawley rats (n=12/sex/dose) tube-fed doses of bovine lactoferrin (purity > 95%) of 0, 200, 600 and 2000 mg/kg bw/d. Two deaths, unconnected to the substance being tested, were observed (one as a result of a mistake in tube feeding, the other from a generalised lymphoma in a female receiving the highest dose of bovine lactoferrin). No change in the animals' body weight or food consumption was observed. At the end of the study, the only statistically significant changes observed in urine analysis were increased sodium, potassium and chloride levels observed only in males receiving the 200 mg/kg bw/d dose. The results of the haematological and biochemical analyses carried out were normal. Measurements of organ weight showed a reduction in absolute and relative weight of the thyroid, only in females receiving the highest dose (2000 mg/kg bw/d). This change in weight was not accompanied by morphological or histological changes of the thyroid and was deemed to be isolated and unconnected to the substance being tested. Histopathological analyses showed cases of pancreatic islet fibrosis in all the male groups, or more specifically in 50% of animals in all the groups treated. The same histopathological changes were observed in 25% of males in the control group. No cases were reported in the females treated with 2000 mg/kg bw/d[2] of bovine lactoferrin, or in the control group of females. The authors conclude that there are no toxic effects and propose a NOAEL of 2000 mg/kg bw/d.

The Dutch committee notes the absence of any specific information regarding the GLP[4] status of the study by Yamauchi et al. (2000). However, it considers that the quality of this study is sufficient to assess safety.

The Dutch committee notes that the variations in urinary parameters are only observed in males and vary over time. They are not accompanied by any abnormal changes in renal tissue or in the blood parameters measured. Similarly to the applicant, the Dutch committee considers as a result that these changes are not significant for toxicological assessment. With regard to the cases of pancreatic islet fibrosis, the Dutch committee considers that they cannot be attributed to treatment with lactoferrin. It explains that such manifestations can occur spontaneously with age, but can also be linked to hormonal imbalances in animals (Imaoka et al., 2007 and 2009).

AFSSA notes that the data available originate from summaries from a dossier relating to a request for GRAS[5] status, without further information on the test protocols used, which makes it difficult to assess their usefulness.
Furthermore, the GLP status of these single-dose and repeated-dose 4-week and 13-week studies is not specified. However, AFSSA considers that the quality of the 13-week study is of an acceptable level.

The above-mentioned 13-week study does not show any statistically significant effects of bovine lactoferrin in the majority of biochemical and histopathological analyses carried out. The only significant effect observed relates to the appearance of cases of pancreatic islet fibrosis. These effects were observed in treated males, but also in untreated controls.

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[1] No Observed Adverse Effect Level.
[2] No measurements carried out in experimental groups receiving intermediate doses (200 and 600 mg/kg bw/d).
[4] Good Laboratory Practices.
[5] Generally Recognised As Safe.

They occurred at a higher intensity and frequency in the experimental groups of males receiving bovine lactoferrin, but without relation to the dose. AFSSA notes the absence of cases of pancreatic islet fibrosis in the tested and control females.

AFSSA considers that the origin of the fibrosis cannot be attributed with any certainty to the age of the animals, since it was a 13-week study and the animals were thus not very old. Furthermore, AFSSA notes that this type of lesion is not commonly reported in toxicological studies on rats. As a result, AFSSA considers that in order to rule out these effects as being "unconnected to the treatment", a history of the frequency with which fibrosis has appeared in animals treated by the laboratory responsible for testing the substance should be provided.

AFSSA notes furthermore that the cases of fibrosis do not show any differences in morphology between animals receiving bovine lactoferrin and those in the control group, and that they are not followed by hyperplasia. AFSSA finds it regrettable that no provision was made for a treatment-free period in certain animals at the end of this study, in order to test the degree of reversibility of the lesions.

In view of all this information, AFSSA considers that the available data make it impossible to draw any conclusions regarding the existence or otherwise of a causal link between the consumption of lactoferrin and the occurrence of pancreatic islet fibrosis in rats.

Conclusion relating to toxicological data:
On the basis of the survey of available toxicological data, the Dutch committee concludes that bovine lactoferrin does not result in adverse effects. Similarly to the applicant, it considers that the effects observed are not attributable to bovine lactoferrin, and confirms the NOAEL of 2000 mg/kg/d tested in the 13-week study.

In conclusion, AFSSA considers that the information available does not allow a NOAEL to be set for bovine lactoferrin.

Allergenic potential:
The applicant refers to several literature reviews which have appeared since 2001 studying the allergenic potential of bovine lactoferrin. On the basis of these data, it concludes that bovine lactoferrin cannot be considered a major cow's milk allergen. It does not rule out the possibility of lactoferrin being one of the allergenic proteins in milk, but in the absence of any oral challenge tests, it believes that this has not been proven.

The Dutch committee recognises that no cause-and-effect relationship has been shown, but notes that IgE antibodies specific to bovine lactoferrin have been found in the blood of patients with an allergy to cow's milk proteins. Thus the possibility of lactoferrin inducing allergies to cow's milk proteins cannot be ruled out, although no experimental data have confirmed this result. As a result, the Dutch committee believes that the presence of cow's milk proteins should be stated on the labels of products containing the NI, all the more so as the NI contains small quantities of other cow's milk proteins (casein, α-lactoglobulin and β-lactoglobulin).

The Dutch committee also notes that it is impossible to make any statement on the development of allergies to cow's milk proteins linked to increasing exposure to bovine lactoferrin. The Dutch committee stresses that short and medium-term clinical studies would not allow an answer to be given to this question, since it requires long-term monitoring. In the opinion of the Dutch committee, the lack of any answer to this question is not, however, a reason to prevent the use of bovine lactoferrin as a novel ingredient. Indeed, every novel protein that an individual consumes entails an inevitable risk of developing an allergy.

In agreement with the Dutch committee, AFSSA considers that if marketing authorisation is granted for the NI, the labelling of the NI and of foods enriched with the NI should clearly state the presence of dairy proteins, in order to inform consumers who are allergic to such proteins.

Regarding involvement of the NI in autoimmune reactions, AFSSA shares the opinion of the Dutch committee, regarding it as improbable that lactoferrin would be likely to induce such a reaction. The suspected involvement of certain dairy proteins in type 1 diabetes has recently been ruled out by EFSA by means of a bibliographic review

(EFSA, 2009). Furthermore, AFSSA does not know of any study having shown bovine lactoferrin to have a harmful effect on immune functions. Nevertheless, as stressed by the Dutch committee, there are few or no studies which have explored the possibility of such effects occurring.

Assessment of safety margins:
The applicant believes that a safety factor of 10 for inter-species differences is unnecessary.

The Dutch committee does not accept this line of reasoning, in particular taking into account the uncertainty relating to methods of absorption in children and adults.
The average consumption estimated in infants (0-1 years) is around 210 mg/kg bw/d, i.e. around 10 times lower than the NOAEL. The Dutch committee considers that this safety margin is acceptable in view of the nature of the NI. It also notes that it is unlikely that infants will exceed this level of consumption. For the general population (except infants), there is a factor of 50 in adults and a factor of 10 in children between 1 and 3 years between the intake at the 95th percentile and the NOAEL. The committee considers that these safety margins are also acceptable.

AFSSA notes that it is impossible to regard the NOAEL as having been clearly established. However, if the value of 2 g/kg bw/d were to be used by the competent authorities in the framework of this assessment, AFSSA believes that it would be necessary to retain the inter-species safety factor, in particular taking into account the lack of data available on humans relating to the methods of digestion and absorption of bovine lactoferrin. A final safety factor of 100 should therefore be applied, as recommended moreover by the FAO[6] (2006). AFSSA thus considers that levels of enrichment should not result in intakes higher than 20 mg/kg bw/d. AFSSA notes that the levels of enrichment proposed by the applicant will however result, on the basis of consumption data from the Dutch population, in an average intake varying from 41 to 124 mg/kg bw/d in different populations of children and adolescents.

4. CONCLUSION

In agreement with the Dutch committee, the French Food Safety Agency believes that the NI is sufficiently characterised and that its manufacturing process does not give rise to any concerns.

AFSSA notes that clinical supplementation studies have not shown any adverse effects linked to the ingestion of bovine lactoferrin in adults, children or infants. However, the aim of these studies was to assess the efficacy, and not the safety, of the NI. AFSSA notes the absence of any in-depth study of a sufficiently large size specifically assessing the long-term safety of bovine lactoferrin.

Furthermore, the toxicology data presented in the 13-week study are insufficient to draw any conclusions regarding the existence or otherwise of a causal link between the consumption of bovine lactoferrin and the occurrence of pancreatic islet fibrosis in rats. Thus a NOAEL cannot be clearly established on the basis of these data.

If a NOAEL of 2 g/kg bw/d should be identified by the competent authorities in the framework of this assessment, AFSSA believes that a safety factor of 100 should be applied. Levels of enrichment should thus be limited in order to guarantee an intake below 20 mg/kg bw/d in all food (total intake via normal food, enriched or otherwise, including food supplements). The levels of enrichment currently proposed by the applicant are likely to result in intakes which greatly exceed this value, in particular for many children and adolescents.

[6] Food and Agriculture Organization of the United Nations.

Furthermore, if marketing authorisation were granted, AFSSA believes that labels for the NI and for food likely to be enriched with the NI should state the presence of dairy proteins.

Finally, AFSSA notes that this assessment does not relate to any possible beneficial effects of bovine lactoferrin which may be claimed, and which will need to undergo specific assessments. AFSSA notes that the data currently available do not provide a sufficient basis to justify supplementary intakes of lactoferrin for humans in good health receiving a varied and balanced diet and with a calorie intake which is sufficient to cover their needs.

The Director General

Marc MORTUREUX

KEY WORDS

Key words: Novel food, dairy protein, transferrin, iron, Regulation (EC) 258/97

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