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Original Submission

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April 6, 2000

Dr. Linda Kahl
Office of Premarket Approval, HFS-200
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, SW
Washington, DC 20204

Dear Dr. Kahl:

We wish to notify you that DMV International has determined that bovine lactoferrin is "generally recognized as safe" ("GRAS") for use as an ingredient in sports and functional foods, and infant formulas. Accordingly, bovine lactoferrin is exempt from the premarket approval requirements of the Federal Food, Drug and Cosmetic Act.

We are hereby submitting the attached document, relied upon by DMV International to make its GRAS determination. As directed by the agency, the information is formatted in accordance with proposal 21 CFR 170.36(c) (62 Fed. Reg. 18937 (April 17, 1997)).

The data and information that serve as the basis for this GRAS notification will be sent to FDA upon request or are available for the FDA's review and copying at reasonable times at the office of Claire Kruger, Ph.D., Senior Science Manager, ENVIRON Corporation, 4350 North Fairfax Drive, Suite 300, Arlington, VA. 22203, telephone: (703) 516-2309, facsimile: (703) 516-2393.

Sincerely,

Claire L. Kruger, Ph.D., D.A.B.T.
Senior Science Manager

cc: V. Frankos
T. Grove
R. Nimmagudda
J. Luedke

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4350 North Fairfax Drive • Suite 300 • Arlington, Virginia 22203 • USA • Tel: (703) 516-2300 • Fax: (703) 516-2345
www.environcorp.com

GENERALLY RECOGNIZED AS SAFE (GRAS) NOTIFICATION FOR BOVINE LACTOFERRIN

Prepared for

DMV International
Fraser, NY

Prepared by

Environ International Corp.
Arlington, Virginia

April 06, 2000

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I. GRAS EXEMPTION CLAIM

A. Name and Address of Notifier

DMV International
1712 Deltown Plaza
Fraser, NY. 13753 USA

Contact: Ram Nimmagudda, Ph.D.
Nutritional Research Manager
Telephone: (607) 746-3082 Ext. 473
Facsimile: (607) 746-8245

B. Name of GRAS Substance

Lactoferrin is the common or trade name for the iron-binding glycoprotein isolated from the bovine species. Published literature may also refer to lactoferrin as red milk protein, lactosiderophilin, ekkrinosiderophilin, or lactotransferrin (Brock, 1980; Naidu, 2000).

C. Intended Use

Lactoferrin has many physiological benefits resulting from its nutrient properties. The manufacturer intends to add bovine lactoferrin to infant formulas and sports and functional foods at the levels listed in Table 1.

TABLE 1
Proposed Levels and Intended Uses of Bovine Lactoferrin

Proposed Applications	Proposed % Substitution by Weight
Infant Formulas	0.1
Sports and Functional Foods	
▪ Wet/Liquid	0.25
▪ Dry/Powdered	0.025

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D. Basis for GRAS Determination

1. Introduction

In this document, we demonstrate that bovine lactoferrin intake resulting from its intended use is safe, and is also Generally Recognized As Safe ("GRAS"), under the Federal Food, Drug, and Cosmetic Act (FDCA or the Act). To accomplish this, first, the safety of bovine lactoferrin intake under its intended conditions of use is established. Then, this intake level is determined to be GRAS by demonstrating that the safety of bovine lactoferrin for its intended use is generally recognized by experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food.

The regulatory framework for establishing whether a substance is GRAS in accordance with Section 201(s) of the FDCA is set forth under 21 CFR §170.30. This regulation states that general recognition of safety may be based on the view of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. A GRAS determination may be made either: (1) through scientific procedures (§170.30(b)); or (2) through experience based on common use in food in the case of a substance used in food prior to January 1, 1958 (§170.30(c)). A scientific procedures GRAS determination under §170.30(b) requires the same quantity and quality of scientific evidence as is required to obtain approval of the substance as a food additive.

Moreover, in addition to requiring scientific evidence of safety, a GRAS determination also requires that this scientific evidence of safety be generally known and accepted. This so-called "common knowledge" element of a GRAS determination consists of the following two components: (1) the data and information relied upon to establish the scientific element of safety must be generally available; and (2) there must be a basis to conclude that a consensus exists among qualified experts about the safety of the substance for its intended use.

The criteria outlined above for a GRAS determination based on scientific procedures, are applied below in an assessment of whether bovine lactoferrin, employed as an ingredient in infant formula (resulting in a mean and 90th percentile consumption of 1.0 and 1.4 g lactoferrin/day, respectively), and sports and functional foods (resulting in a mean and 90th percentile consumption of 1.0 and 2.1 g lactoferrin/day, respectively), is GRAS. The lactoferrin intake from infant formula is intended to supplement the normal infant dietary intake of up to 245 mg/day, derived from the whey protein contained in infant formula. This results in a total mean daily consumption of up to 1.3 g lactoferrin/day for infants. The lactoferrin intake from sports and functional foods consumed by children, teens or adults, is also intended to supplement the normal dietary

intake from dairy sources of up to 75 mg/day at the 90th percentile of intake. This results in a total mean daily consumption of lactoferrin of up to 1.1 g/day.

Once bovine lactoferrin is determined to be GRAS for its intended use, it is permitted to be used for that purpose because it is not (by definition) a food additive, and therefore does not require promulgation of a specific food additive regulation under 21 CFR prior to marketing.

2. Safety of Bovine Lactoferrin for its Proposed Use

a) Traditional Approach for Evaluating the Safety of Food Additives

The regulatory criterion by which the safety of a food additive is judged is that "there is a reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use" (21 CFR § 170.3(i)). This regulation specifies that three factors be considered in determining safety. These are:

- The probable consumption of the substance and of any substance formed in or on food because of its use;
- the cumulative effect of the substance in the diet, taking into account any chemically or pharmacologically related substance or substances in such diet;
- safety factors which, in the opinion of experts qualified by scientific training and experience to evaluate the safety of food and food ingredients, are generally recognized as appropriate.

After consideration of these factors, the FDA usually establishes an acceptable daily intake (ADI) for the additive. The ADI represents the maximum amount of the additive that can be safely consumed by humans on a daily basis for a lifetime. The FDA has specified that an ADI is usually established by application of a safety factor of at least 100 to the lowest NOAEL identified in the most sensitive animal species studied. The FDA also considers evidence that might justify use of a different safety factor (21 CFR § 170.22). Except where evidence is submitted which justifies use of a different safety factor, a safety factor in applying animal experimentation data to man of 100 to 1 is used; that is, tolerance for the use of a human food ingredient will not exceed 1/100th of the maximum amount demonstrated to be without harm to experimental animals.

b) Approach for Evaluating the Safety of Macroingredients

Macroingredients are a class of food additives that are intended to be replacements for conventional macroingredients such as fats, proteins, and carbohydrates, and thus are intended for use at relatively high levels in food (FDA 1993). Macro-ingredients have the potential to be consumed in gram quantities per day (whereas traditional food additives are generally consumed at levels below 1 gram/day) because they are intended to replace energy-dense traditional food constituents that form a substantial part of the diet (Borzelleca 1992). There is a current trend toward increased consumption of macroingredients because of a public health emphasis on reducing both total caloric intake and dietary fat intake, and because of consumer demand for more and varied products with fewer calories and less fat (Rulis 1990; FDA 1991). Thus, both the food industry and the FDA have intensified their efforts to develop a more appropriate approach to assess the safety of macroingredients.

One of the key features that differentiates the safety assessment approach for macroingredients from that for traditional food additives is the potentially large human exposure to macroingredients. If macroingredients are tested in animal studies at substantial multiples of the anticipated human exposure, similar to what is done for the testing of traditional food additives, spurious results would occur. These results would not reflect toxicological effects due to the macronutrient, but rather perturbations resulting from physiological or nutritional imbalances created by the large doses of the macronutrient administered (Borzelleca 1992; IFBC 1990; FDA 1993; Verschuren 1988; WHO 1987; Feron et al. 1990). Therefore, macroingredients require an alternative safety assessment approach that utilizes information about the physical, chemical, and biological properties of the macronutrient.

These properties typically include the digestibility, absorption and metabolic fate of the macronutrient. This information allows for the prediction of potential target organs and toxic effects (Munro 1994). Initial short-term toxicity testing with rodents can be used to identify any unanticipated systemic toxicity. However, testing should then proceed to more appropriate models, including humans, to confirm safety and to assess specific endpoints such as palatability, nutritional status and tolerance (Borzelleca 1992; Munro 1994). Exposures to the macronutrient in human trials should be at multiples of the estimated daily intake. Human tolerance to the macronutrient can then be used as the basis for identifying an acceptable daily intake (Borzelleca 1992). Characteristics such as the

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similarity of a macronutrient to a traditional food, metabolic conversion into normal body constituents, and lack of overt toxicity allow the use of human studies, supported by other data establishing safety, to be used in the development of an acceptable daily intake or acceptable intake level (AIL) for a macroingredient.

Therefore, the traditional ADI/safety factor approach outlined in the previous section is generally not appropriate for use with food additives that will be consumed in relatively large quantities, such as bovine lactoferrin. For these macroingredients an alternative approach is generally needed to establish an AIL. The FDA's Red Book II (FDA 1993) states:

> The common characteristic of macro-additives is that they will be consumed in large quantities compared to conventional food additives and, as a consequence, they will present testing problems that require "customized" approaches. For example, it may not be feasible to calculate safety factors in the conventional way, that is, as a fraction of the highest oral dose that has no adverse effects in animals. Other means of providing margins of safety for macro-additives will have to be used; these may include information derived from metabolic, pharmacokinetics, and human clinical studies.

Therefore, a safe intake level for bovine lactoferrin, used as a macroingredient, will be derived by employing an AIL approach, rather than the traditional safety factor approach. This AIL approach for bovine lactoferrin is described in greater detail in the following section.

c) Acceptable Intake Level (AIL) for Bovine lactoferrin

An extensive database, consisting of both animal and human exposure and safety data, is available for determination of the safety of bovine lactoferrin use as an ingredient in infant formula, sport or functional food. These data will be used to determine the AIL for the use of bovine lactoferrin in these products.

Bovine lactoferrin has been consumed by humans for thousands of years as a naturally occurring protein found in milk. Consumption of bovine lactoferrin through dairy sources has been estimated to be 73, 75, and 50 mg/day at the 90th percentile of intake for children, teens and adults, respectively. In addition to dairy sources, lactoferrin is currently marketed in the U.S. as a dietary supplement. Consumption of lactoferrin from this source ranges from 10 to 1200 mg/day.

Infants who are breast-fed consume human lactoferrin which is a natural constituent of human milk. Human milk contains from 1 to 4.2 mg/ml, resulting in a daily consumption of 1 to 4.2 g/day, assuming a daily consumption of 1 liter. As a comparison, infants who consume infant formula derived from cow protein only consume up to 245 mg/day of bovine lactoferrin, derived from the whey protein component. There are several infant formulas sold outside the U.S. that contain supplemental lactoferrin. The supplemental lactoferrin adds up to 107 mg/day in addition to the maximum of 245 mg/day derived from whey protein. Therefore, infants consuming these formulas may consume up to 352 mg/day bovine lactoferrin.

A great deal is known about the fate of ingested human and bovine lactoferrin in the body. Unlike many dietary proteins, lactoferrin is only slowly degraded in the gut, as demonstrated in the piglet, baby monkey, and human newborn. Studies in human infants demonstrate that considerable quantities of intact human lactoferrin are excreted by the breast-fed infant. Higher amounts are excreted during the early weeks of life and the quantity excreted is progressively smaller during successive weekly measurements. During the first weeks of life, approximately 2 to 6% of the lactoferrin consumed by the infant is excreted in the feces per day; however, by 4 months, less than 2% of the lactoferrin intake is excreted. Increased proteolysis of the lactoferrin takes place as the infant ages. The lactoferrin excreted in the feces is intact. Compared with other whey proteins, the relatively large amounts of lactoferrin excreted intact in the feces from infants, along with the finding that no other major whey proteins are detected in the feces, reinforces the fact that lactoferrin is protected from degradation.

Two studies of lactoferrin excretion in infants fed formula containing supplemental bovine lactoferrin indicated that, as expected, babies fed formula containing bovine lactoferrin excreted significantly more lactoferrin in their stools than babies fed the basic formula. The levels excreted, however, were significantly lower than levels excreted from breast fed babies. Most of the lactoferrin from the formula appeared to have been broken down.

In contrast to these findings, however, a third study of the fate of bovine lactoferrin and human lactoferrin fed to healthy babies aged 3 days to 2 months showed that bovine lactoferrin was less degraded than the human forms and total excretion per day of the intact protein was greater. Quantitative determination of human copro-lactoferrin from birth through a period of up to 3 weeks showed that the daily elimination decreased from 25 to 5 mg. The amount of copro-lactoferrin

corresponding to the endogenous secretion (biosynthesis by the intestinal tract) was calculated to be from 0.5 to 1 mg/day.

Intact lactoferrin has also been found in the urine of human milk-fed preterm infants. Because serum levels of lactoferrin in both preterm and term infants do not vary with diet (i.e., human milk vs. bovine milk based formula), studies were conducted to determine the origin of the lactoferrin in urine. It was determined that nearly all of the intact lactoferrin in the urine is of maternal origin

The mean plasma level of lactoferrin in adults is 122 ± 40 μg/L; in term infants up to the age of 21 days, the mean lactoferrin level is 385 ± 113 μg/L. The difference in the means between adults and infants is statistically significant. There were no significant differences between the means of breast fed and formula fed infants. No significant differences in plasma levels were found between adults and infants at 15 weeks of age.

The plasma lactoferrin concentration in adults is derived from neutrophils. Lactoferrin derived from breast milk, given to adults, is rapidly cleared from the circulation, being avidly sequestered by the liver and spleen. The sequestered lactoferrin is promptly catabolized. The iron, however, is not excreted in the urine, but rather retained and slowly transferred to the bone marrow, where it is subsequently incorporated into developing erythrocytes.

The safety of bovine lactoferrin, similar in specifications to the bovine lactoferrin from DMV International, which is the subject of this GRAS determination, was assessed in an acute toxicity study, a 4-week oral toxicity study, a thirteen-week oral toxicity study and an Ames assay.

Lactoferrin exhibited low acute oral toxicity. A single oral dose of 1,000 or 2,000 mg/kg bovine lactoferrin or bovine iron-saturated lactoferrin resulted in no adverse effects or deaths. Based on these results, the lethal dose of lactoferrin exceeds 2,000 mg/kg.

In the 4-week toxicity study, male and female Sprague-Dawley rats were exposed by oral intubation to 200, 600, or 2,000 mg/kg/day bovine lactoferrin once daily for 4 weeks (28 days). Administration of 200, 600, and 2,000 mg/kg/day bovine lactoferrin to male and female rats resulted in no deaths or treatment-related changes in body weight, food consumption, organ weight, ophthalmology, hematology, blood chemistry, urinalysis, or gross pathology and histology examinations. Therefore, the no-observed-adverse-effect ("NOEL") level of bovine lactoferrin was estimated to be in excess of 2,000 mg/kg/day.

In a thirteen-week toxicity test, male and female Sprague-Dawley rats, 4 weeks of age, were exposed to 200, 600, or 2,000 mg/kg/day bovine lactoferrin by

oral intubation once daily, seven days a week for 13 weeks. Bovine lactoferrin administered by oral intubation to rats for 13 weeks did not result in significant test material related changes in the appearance, general condition, body weight, food consumption, ophthalmology, hematology, blood chemistry, gross pathology, or histology of the animals. Thus, under the conditions of this study, the NOEL for bovine lactoferrin was estimated to be in excess of 2,000 mg/kg/day.

Lactoferrin was examined for mutagenic potential in a reverse mutation assay. It did not cause a positive response in any of the tester strains in the presence or absence of S9 activation at concentrations up to 5,000 μg/plate. Thus, under the conditions of the study, lactoferrin was not found to be genotoxic in the *Salmonella typhimurium* reverse mutation assay or the *Escherichia coli*/mammalian microsome reverse mutation assay.

Humans have been exposed to bovine lactoferrin in numerous clinical trials that were designed to look at effects on either iron absorption or modulation of microflora or infection. In studies of infants, dose levels used ranged from 1.4 mg/day (0.3 mg/kg/day) to 2.9 g/day (1.0 g/kg/day) and study durations were from 11 days to 5 months. In studies of adults, dose levels used ranged from 100 mg/day (1.7 mg/kg/day) to 3.6 g/day (60 mg/kg/day) and study durations varied from a single dose in one study to 8 weeks. Despite the fact that the studies were not designed specifically to look for issues of tolerance to ingestion of lactoferrin, no adverse health effects as a result of lactoferrin intake were reported in any of the studies. There are also no indications of subjects discontinuing treatment due to adverse reactions.

In conclusion, animal studies indicate that there are no adverse effects related to lactoferrin consumption at levels up to 2,000 mg/kg/day given for up to 13 weeks. *In vitro* studies provide no evidence of mutagenic potential.

Background exposure to lactoferrin by children, teen and adults, from dairy products ranges from 50 to 75 mg/day at the 90th percentile of consumption and from dietary supplements ranges from 10 to 1,200 mg/day.

Levels of lactoferrin present in human milk indicate that the concentration can range from 1 to 4.2 mg/ml. This would result in lactoferrin intake of 1 to 4.2 g/day by infants, assuming an intake of 1 L of human milk per day. Consumption of infant formula sold in the U.S. results in intakes of lactoferrin up to 245 mg/day from the whey protein; infant formulas supplemented with lactoferrin and marketed outside the U.S. provide up to 352 mg/day of lactoferrin to the infant.

Human clinical trials have exposed infants to lactoferrin doses of up to 2.9 g/day and adults to doses up to 3.6 g/day. Human clinical data indicate that bovine lactoferrin intakes in adults at levels of 3.6 g/day and below and in infants at levels of 2.9 g/day and below are well tolerated and without reported adverse health effects.

The AIL for bovine lactoferrin, for use as an ingredient in sports or functional foods can be derived from the long history of safe consumption of lactoferrin from dairy sources and dietary supplements and the lack of adverse findings in clinical trials at doses up to 3.6 g/day. The AIL is therefore proposed to be less than or equal to 3.6 g/day for the general population with the exception of infants under 1 year.

The safe level of exposure by infants less than 1 year to bovine lactoferrin from infant formula can be derived from the natural levels of the human protein present in human milk (1 to 4.2 g/day), supporting safety information derived from history of exposure to lactoferrin in infant formulas, and the results from clinical trials of bovine lactoferrin at dose levels up to 2.9 g/day. It is reasonable to suggest that a safe level of exposure to lactoferrin should be one that is within the normal background exposure from human milk. Therefore, the AIL for infants less than 1 year old is conservatively proposed to be 2 g/day.

d) Estimated Daily Intake (EDI) of Bovine Lactoferrin from Proposed Use

Bovine lactoferrin is proposed for use as an ingredient in sports and functional foods (proposed substitution by weight of 0.25% for wet/liquid products and 0.025% for dry/powdered products), and infant formulas (proposed substitution by weight of 0.1%).

The proposed EDI of bovine lactoferrin through its use in sports and functional foods is 1.0 and 2.1 g/day at the mean and 90th percentile of consumption. (The consumption of these foods is intended to supplement normal dietary intake of up to 75 mg/day from dairy sources, resulting in a total mean daily intake of up to 1.1 g/day.)

The proposed EDI of bovine lactoferrin through its use in infant formula is 1.0 and 1.4 g/day at the mean and 90th percentile of consumption. (The consumption of supplemental lactoferrin from formula is in addition to the lactoferrin available from the whey protein which can result in consumption of up to 245 mg/day. Therefore, total mean daily intake of lactoferrin from infant formulas would be up to 1.3 g/day.

e) Safety Evaluation Following Chronic and Acute Intake of Bovine Lactoferrin

Evaluation of the safety of bovine lactoferrin is accomplished through a review of the extensive database on the safety of bovine lactoferrin, including history of human exposure, animal and human studies, and a comparison of the current AIL to the EDI of bovine lactoferrin. If the EDI is less than (or approximates) the AIL, then the proposed use can be considered to be safe.

The proposed EDI of bovine lactoferrin through its use in sports and functional foods is 1.0 and 2.1 g/day at the mean and 90th percentile of consumption. (The consumption of these foods is intended to supplement normal dietary intake of up to 75 mg/day from dairy sources, resulting in a total mean daily intake of up to 1.1 g/day.) The proposed EDI of bovine lactoferrin through its use in infant formula is 1.0 and 1.4 g/day at the mean and 90th percentile of consumption. (The consumption of supplemental lactoferrin from formula is in addition to the lactoferrin available from the whey protein which can result in consumption of up to 245 mg/day. Therefore, total mean daily intake of lactoferrin from infant formulas would be up to 1.3 g/day.)

Human exposure to lactoferrin and data from human and animal studies, indicate that bovine lactoferrin intake at levels of up to 3.6 g/day in the general population and intake at levels up to 2.0 g/day in infants are well tolerated and without reported adverse health effects. It can thus be concluded that the AIL for supplemental consumption of bovine lactoferrin as an ingredient in sports and functional foods up to 3.6 g/day and the AIL for use in infant formulas is up to 2 g/day. The EDI from the proposed use of bovine lactoferrin in sports and functional foods of 1.0 g/day (with a total daily intake of 1.1 g/day) is below the AIL of 3.6 g/day. The EDI from the proposed use of bovine lactoferrin in infant formula of 1.0 g/day (with a total daily intake of 1.3 g day) is below the AIL of 2.0 g/day. The consumption of bovine lactoferrin from the proposed uses and levels is therefore concluded to be safe.

3. General Recognition of the Safety of Bovine Lactoferrin

This document presents the information used to determine the GRAS status of bovine lactoferrin for use as an ingredient in sports and functional foods, and infant formula. This determination of safety is based upon a critical review of both toxicological and clinical safety studies as well as supporting information derived from a history of exposure.

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A review of the following information was completed in the evaluation of the GRAS status of bovine lactoferrin: background information (i.e., composition and uses), consumer exposure data, and biological studies in both animals and humans. The sources of the reviewed information included pertinent studies and literature provided by DMV International or identified in literature searches conducted through online bibliographic retrieval systems, including Medline and Dialog.

In the previous section, the proposed uses of bovine lactoferrin as an ingredient in sports and functional foods, and infant formulas were determined to be safe through scientific procedures. Under 21 CFR §170.30, general recognition of this safety requires that the scientific data and information upon which the determination of safety rests must ordinarily be published, but may be corroborated by unpublished studies and other data and information. The scientific data and information on which the safety determination of bovine lactoferrin is based are available in the published literature or are otherwise publicly available to experts qualified by training and experience to evaluate the safety of food and food additives. Thus, the data reviewed meet the scientific procedure element required for all GRAS determinations.

Determination of the GRAS status of bovine lactoferrin as a food ingredient has been made through the deliberation of experts, Dr. Vasilios H. Frankos and Dr. Claire L. Kruger, scientists qualified by training and experience to evaluate the safety of food ingredients. These experts have carefully reviewed all the available data on the metabolism and short-term and long-term toxicity of bovine lactoferrin, human exposure and tolerance to bovine lactoferrin, and human clinical safety studies evaluating safety of administered intakes of bovine lactoferrin and have concluded that:

> There is no evidence in the available information on bovine lactoferrin that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when it is used at levels that are now current or that might reasonably be expected from the proposed applications. Bovine lactoferrin is GRAS for use as an ingredient in sports and functional foods, and infant formulas, as proposed by DMV International.

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It is their opinion that other qualified and competent scientists reviewing the same publicly available data would reach the same scientific conclusion. Therefore, bovine lactoferrin, to be used as an ingredient in sports or functional foods, and infant formulas is GRAS, at an intake level up to and including 3.6 g/day in sports or functional foods and up to and including 2.0 g/day in infant formulas. Because bovine lactoferrin is GRAS for its intended use, it is excluded from the definition of a food additive, and thus may be marketed for this use without the need to promulgate a specific food additive regulation under 21 CFR.

E. Availability of Information

The data and information that serve as the basis for this GRAS notification will be sent to FDA upon request or are available for the FDA's review and copying at reasonable times at the office of Claire Kruger, Ph.D., Senior Science Manager, ENVIRON Corporation, 4350 North Fairfax Drive, Suite 300, Arlington, VA. 22203, telephone: (703) 516-2309, facsimile: (703) 516-2393.

II. DESCRIPTION OF SUBSTANCE

A. Physical and Chemical Composition

Bovine lactoferrin has a molecular weight of approximately 75 to 80-kDa (Table 2). The isoelectric point (pI) of lactoferrin measures 8.2 to 8.9 by the chromatofocusing method (Shimazaki et al. 1993) and 9.5 to 10 by the isoelectric focusing method (Yoshida and Xiuyun 1991). Desaturated lactoferrin (apo-form) has an absorbance of 12.7 when measured at 280 nm (Aisen & Leibman 1972). The absorbance of iron-saturated lactoferrin (holo-form) is 0.400 at 470 nm. Holo-lactoferrin is more resistant to thermal denaturation than desaturated apo-lactoferrin (Paulsson et al. 1993).

Bovine lactoferrin consist of a single polypeptide chain of about 708 amino acids (Goodman and Schanbacher 1991). The sequence has been determined by cDNA and protein sequencing (Mead and Tweedie 1990). The protein contains intramolecular disulfide bonds but is absent of free sulfhydryl groups. Lactoferrin is glycosylated at two different sites by N-linked glycans of the N-acetyllactosaminic type. These glycans are characterized by α-1,3-linked galactose residues in the terminal nonreducing position. Unlike human lactoferrin, bovine lactoferrin also contains glycans of the oligomannosidic type (Spik et al. 1988; Spik et al. 1994)

The polypeptide chain consists of two separate lobes (Figure. 1) that are linked by a three-turn α-helix (Nuijens et al. 1996). These lobes, designated N- and C- terminal, are similar in amino acid sequence (Lönnerdal and Iyer 1995). The tertiary structure of this glycoprotein has two iron-binding sites, giving it the capability to bind two molecules of iron per molecule of protein. Carbohydrate analysis shows that each molecule of bovine lactoferrin contains 1 residue of terminal sialic acids, 10 to11 residues of N-acetylglucosamine, 5–6 residues of galactose and 15 to 16 residues of mannose (Castellino et al. 1970).

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TABLE 2 Physical-Chemical Properties of Lactoferrin		
Property	**Bovine Lactoferrin**	**Reference**
Molecular mass		
Sedimentation co-efficient	77,200 ± 1,300	Castellino et al. 1970
SDS-PAGE	76,000 ± 2,400	Querinjean et al. 1971
Iron titration	78,500	Aisen & Leibman 1972
Isoelectric		
Chromato focusing	8.2-8.9	Shimazaki et al. 1993
Isoelectric focusing	9.5-10.0	Yoshida and Xiuyun 1991
Absorption spectra		Aisen & Leibman 1972
Apo-form at 280 nm	12.7	
Holo-form at 470 nm	0.400	
Glycosylation	Low	Metz-Boutigue et al. 1984
Protease sensitivity	High	Brines & Brock 1983
IgA-complexes	Absent	Watanabe et al. 1984
Iron-binding		Aisen & Leibman 1972
Equilibrium dialysis (K1 x 10^{-4})	3.73	
Thermal denaturation		Paulsson et al. 1993
Apo-LF denaturation (Tmax: °C)	71 ± .3 & 90 ± .3	
Apo-LF enthalpy (ΔH_{cal}: J/g)	12 ± .4 & 2 ± .5	
Holo-LF denaturation(Tmax: °C)	65 ± .3 & 93 ± .3	
Holo-LF enthalpy (ΔH_{cal}: J/g)	2 ± 1 & 37 ± 1	
Adopted from Naidu 2000		

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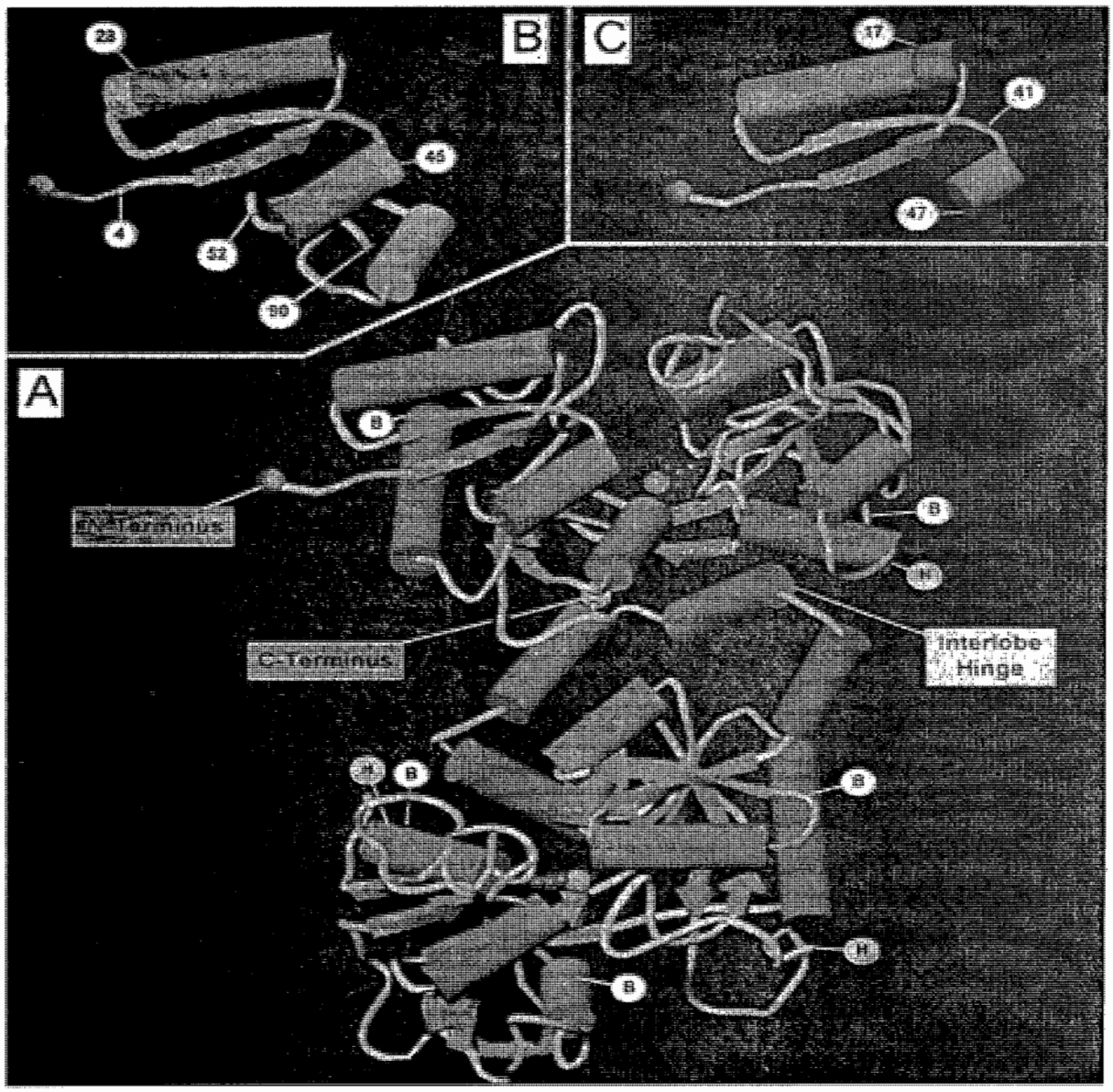

Fig. 1. Diagram of the linear sequence and three-dimensional structure of human lactoferrin, with secondary structure features represented by cylinders (helices) and twisted arrows (beta-strands). The linear sequence diagram of lactoferrin shows the relative sequence location of the bactericidal peptides (lactoferricins H and B) and lymphocyte receptor binding regions, based on hLf sequence. The three-dimensional structure of human lactoferrin (A) shows iron (orange sphere) and coordinating anion (purple spheres) in the iron-binding site between the two domains (I & 11) within each lobe. The N-terminus is indicated by a blue sphere. The C-terminus (green sphere) and interlobe hinge helix (labeled) are shown for the full structure in (A). Alpha-helical structure is shown as red cylinders; P-strands as blue arrows. The glycosylation sites for human (H, gray) and bovine (B, white) lactoferrin are indicated as attached circles. Outsets highlight the N-terminal bioactive region showing: (B) the primary large (1-90) and the minimum (hLF 4-52, darker colors) lymphocyte receptor binding regions with the two putative receptor binding subregions (green) spanning residues 28-45, and (C) the bactericidal peptide regions of human (hLf 1-47, lactoferricin H) and bovine (bLf 17-41, lactoferricin B, shown in orange and green) lactoferrins superimposed onto the hLF structure. Diagram adopted from Nuijens et al. 1996.

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B. Manufacturing Process

1. General Description of the Production Process

Bovine lactoferrin is extracted from cow's milk or milk derivatives such as whey. Raw milk is heated to 50°C, separated into skim milk and cream then pasteurized at 70°C for at least 20 seconds. Whey or pasteurized skim milk is subjected to microfiltration in order to reduce microbial load and fat content. The filtrate is then passed over a cation exchanger at a high velocity and high liquid load. Lactoferrin, which is adsorbed into the resin, is eluted and then filtered through various systems before being spray dried. Figure 2 identifies each step in the production process. A detailed explanation of the process is disclosed in US Patent No. 5,596,082 (Appendix A).

2. Processing Aids and Chemicals

Processing chemicals and aids used in the production of bovine lactoferrin are listed in Table 3. Process chemicals consist of food grade sodium chloride, phosphoric acid (75%), disodium phosphate, and demineralized water. Processing chemical comply with 21 CFR § 182.1(a), 182.1073, and 182.6290, respectively. Process aids are also FDA compliant (comply with 21 CFR § 170.30(b), 177.2910 (a), 177.2240, and 177.2510) and include SP Sepharose Ion Exchange Media, microfilters and ultrafilters. Safety-information sheets for the processing chemicals are located in Appendix B.

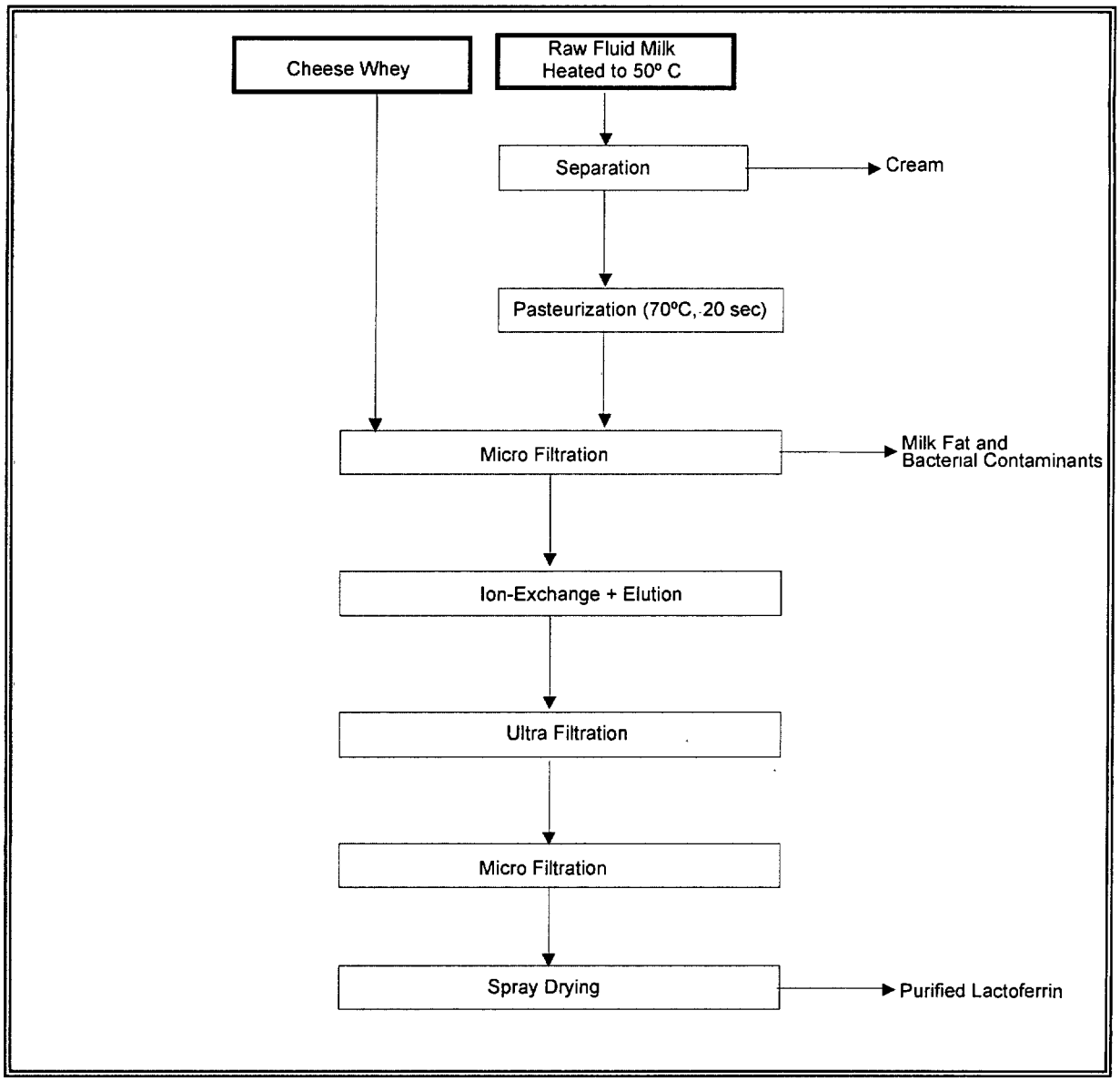

Figure 2. Diagram of the Production Process of Bovine Lactoferrin

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TABLE 3 Processing Aids and Chemicals Used in the Production of Lactoferrin.	
Process Aid or Chemical	**Manufacturer**
Demineralized water	DMV International
Disodium phosphate	CFB – Chemische Fabrik Budenheim Rudolf A Oetker
Phosphoric acid (75%)	Caldic Nederland BV
Sodium chloride	Centrale Aankoop FNZ
SP Sepharose Ion Exchange Media	Pharmacia
Membralox Ceramic Membrane	SCT
HFK-131 (PES) Spiral Membrane	KOCH Membrane Systems
Durapore - PVDF	Millipore

3. Finished Product Specifications

The manufacturing specifications for bovine lactoferrin appear in Table 4. The product consists of 93.0% protein, 6% moisture and 1% ash. Lactoferrin makes up 95% of the protein content. Typical pH range of the finished product is between 5.5 to 6.5 with a water activity (a_w) of 0.2. Complete solubility is achieved when 2 g of lactoferrin are added to 100 ml of 20°C water. The iron binding capacity of the final product is ≥70%. Batch analyses from 3 separate lots of bovine lactoferrin are provided in Appendix C.

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TABLE 4 Manufacturing Specifications for Bovine Lactoferrin	
Chemical/Physical/Microbial Specifications	**Specifications**
Protein	≥ 93.0%
• Lactoferrin	≥ 95.0%
Moisture	≤ 6.0%
Ash	≤ 1.0%
pH (2%, 20°C)	5.5-6.5
a_w – not measured on a routine basis	.2
Solubility	
• In water (2%, 20°C)	≥ 100%
• Transmittance, 2% sol., 600nm	≥ 80%
Iron Binding	
• Spectrophotometric method at 465 nm on solids	≥ 70%
Foreign matter (10g)	Absent
Heavy metals (as Pb)	≤ 1 mg/kg
Lead	<0.5 mg/kg
Arsenic	<1.0 mg/kg
Cadmium	<0.05 mg/kg
Mercury	<0.05 mg/kg
Copper	<2.0 mg/kg
PCB's [1]	<0.1 mg/kg
Pesticides [2]	<0.1 mg/kg
Antibiotics	<0.005 IU/ml
Alfatoxin MI	<0.5 μg/kg
Radioactivity [3]	<5.0 Bq/kg
Standard plate count	≤ 1000/g
Enterobacteriaceae	≤ 10/g
Salmonella 2 x 1 g	Negative/50g
S. aureus	Negative
Total yeast and mold	≤ 10/g

[1] PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153, PCB 180

[2] Dieldrin, heptachlor epoxide, hexachlorobenzene, α-hexachlorocyclohexane, β- hexachlorocyclohexane, lindane, DDT-total

[3] Cs 134 + Cs 137

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4. Pathogenic Bacteria

Bovine lactoferrin was also analyzed for the presence of *Listeria monocytogenes*, *Clostridium perfringens*, *E. coli*, Brucellosis *and Yersinia enterocolytica* because they are potential contaminants of raw milk. No pathogenic bacteria were detected (Table 5). The laboratory results can be found in Appendix C.

Table 5
Pathogenic Bacteria Analysis of Bovine Lactoferrin

Microorganism	Result
Listeria monocytogenes	Not detectable
Clostridium perfringens	Not detectable
Escherichia coli	Not detectable
Brucellosis	Not detectable
Yersinia enterocolytica	Not detectable

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III. USE AND CONSUMER EXPOSURE

A. Historical Background Exposure

1. Human Lactoferrin

Human lactoferrin (hLF) is a naturally occurring protein that is found mainly in external secretions, such as breast milk, mucosal surfaces and in the secondary granules of the neutrophils (Chierici et al. 1994; Nuijens et al. 1996). It is synthesized by the epithelium and neutrophils and is released by these cells in response to inflammatory stimuli (Lönnerdale and Iyer 1995). Lactoferrin present in mother's milk may be involved in the host resistance mechanism of breast-fed infants (Bezkorovainy, 1977; Goldman and Smith 1973). It has been shown to inhibit the growth of a number of microorganisms (Chierici et al. 1994; Nuijens et al., 1996); play a role in intestinal iron uptake and excretion (Lönnerdale and Iyer 1995); and promote the growth of intestinal epithelial cells (Nuijens et al. 1996).

Johansson (1960) isolated lactoferrin from human breast milk and determined it was very similar to the serum iron-binding protein transferrin. The complete amino acid sequence (703 amino acid residues) of human lactoferrin was determined by Metz-Boutique (1984). The single polypeptide chain is folded into two lobes. Each lobe contains a single iron-binding site and a single glycosylation site. The protein contains 5 to 6% carbohydrate, mainly galactose, mannose, fructose, N-acetylglucosamine and sialic acid (Jenness 1979; Mazurier 1974).

a) Concentration in Human Milk

Human colostrum, which is expressed during the first 3 days postpartum, contains the highest concentration of lactoferrin, 3.1 to 0.58 mg/ml. Human milk also contains a relatively large amount of lactoferrin with a concentration ranging between 1 and 4.20 mg/ml (Table 6) with a mean of approximately 2.0 mg/ml. The changes in lactoferrin concentration during the different stages of lactation were determined by Lönnerdal et al. (1976b) and are presented in Table 7. As lactation period increases, the concentration of lactoferrin in the milk decreases.

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TABLE 6 Concentration of Lactoferrin (mg/ml) in Human Colostrum and Milk		
Human Colostrum mg/ml	**Human Milk mg/ml**	**Reference**
10.58	1.74	Fransson and Lönnerdal 1980
3.3	1.67	Hambraeus et al. 1978
	1-2	Hennart et al. 1991
6.7	2.6	Hirai et al. 1990
	1.55	Lönnerdal et al. 1976a
	4.2	Masson and Heremans 1971
3.1		Mathur et al. 1990
	2.1	Nagasawa et al. 1972
	1.97-3.20	Pamblanco et al. 1986
	1-3	Rudloff and Kunz 1997
	> 2	Tomita M. 1999
	1.4-2.6	Velona et al. 1999

TABLE 7 Changes in Lactoferrin Concentration (mg/ml) in Human Milk during Lactation				
Months postpartum				**Reference**
0-0.5	**0.5-1.5**	**1.5-3.5**	**3.5-6.5**	
3.53	1.94	1.65	1.39	Lönnerdal et al. 1976b

b) Comparison of Human and Bovine Lactoferrin

The similarities in and differences between bovine and human lactoferrin have been studied extensively. The amino acid composition (mole %) of the two proteins is shown in Table 8. The nucleic acid sequence and the amino acid sequence of bovine lactoferrin are homologous (77% and 68%, respectively) with published sequences for human lactoferrin (Lönnerdal and Iyer 1995). The two also share extensive homology in disulfide bonding, the lack of free sulfhydryls, and in the secondary structure (Crichton 1990).

Baker et al. (1994) used high resolution crystallograph to study the three-dimensional structure of various forms of lactoferrin. The most striking difference between human and bovine lactoferrin was in the relative orientation of the two lobes. If the N-lobes of the two lactoferrins were superimposed, one would have to rotate the C-lobes approximately 12° before they would line up. The authors noted that only one of the four glycosylation sites on bovine lactoferrin was also found on human lactoferrin.

Wang et al. (1984) studied the chemical and immunochemical properties of human and bovine lactoferrin. Results showed that bovine lactoferrin could only be differentiated from human lactoferrin by immunochemical analysis. Bovine lactoferrin was shown to have an iron content approximately four-fold higher than that of human lactoferrin. Since lactoferrin bacteriostatic properties are inversely related to the extent of iron saturation, the authors concluded that bovine lactoferrin is less effective as an anti-infectious reagent than human lactoferrin.

TABLE 8
Amino Acid Composition of Bovine and Human Lactoferrin

Amino Acid	Bovine Lactoferrin mole %	Human Lactoferrin mole %
Lysine	7.75	6.44
Histidine	1.38	1.19
Arginine	5.82	5.49
Aspartic acid	9.72	10.84
Threonine	5.22	5.32
Serine	5.02	6.70
Glutamic acid	9.84	10.41
Proline	4.46	4.30
Glycine	7.43	7.71
Alanine	9.79	8.88
Half-cystine	3.84	4.49
Valine	6.64	7.28
Methionine	0.38	0.21
Isoleucine	2.62	2.78
Leucine	10.58	8.23
Tyrosine	3.46	2.79
Phenylalanine	4.32	3.96
Tryptophan	1.67	2.94

Source: Wang et al. 1984

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2. Bovine Lactoferrin

Milk from cattle (*Bos taurus*) has been consumed by the human population for centuries. Bovine milk is composed of a complex mixture of lipids, proteins, carbohydrates, vitamins, and minerals. The average composition of milk consist of 86.6% water, 4.1% fat, 3.6% protein, 5.0% lactose, and 0.7% ash (Swaisgood et al 1985).

Milk protein is a combination of caseins and whey proteins. Their relative amounts are shown in Table 9. Caseins account for 79% (27 mg/ml) of the total milk proteins. The remaining 21% (7.1 mg/ml) is whey protein. Lactoferrin, which is a whey protein, accounts for 3% (0.1 mg/ml) of the total protein in bovine milk (Barth and Behnke 1997).

Lactoferrin was first isolated from bovine milk by Groves in 1960. Since then several investigators have isolated and characterized this protein (Goodman and Schanbacher 1991; Magnuson et al. 1990; Mead and Tweedie 1990; Rejman et al. 1989). Bovine lactoferrin has been shown to inhibit the growth of a number of microorganisms including *Listeria monocytogenes* (Wakabayashi et al. 1992).

TABLE 9
Concentration of Proteins in Milk

Components	mg/ml Milk	%
Milk Proteins	34.1	100
• Caseins	27.0	79
• Whey Proteins	7.1	21
▪ Lactoferrin	0.1	0.3

[1] Adapted from Barth and Behnke 1997.

a) Concentration in Bovine Milk and Milk Products

Just as in humans, the highest concentration of lactoferrin is found in the cow's colostrum (2 to 5 mg/ml) which is expressed the first few days postpartum (Table 10). Milk, by definition is a lacteal secretion that is free of colostrum (21 CFR § 182.6290). The average lactoferrin content of milk is approximately 0.1 mg/ml; which means an 8-oz glass of milk would contain about 23 mg of lactoferrin.

Milk must be thermally processed by High Temperature Short Time (HTST) pasteurization or ultra-pasteurization (UP) in order to ensure consumer safety (FDA, 1997). Saito et al. (1994) showed that lactoferrin was easily denatured at ≥

pH 6 when heated at 80 to 120°C for 5 min. The time-temperature relation used in this experiment exceeds the guidelines for both HTST (72°C for 15 sec) and UP (138°C for 2 sec) treatments. Therefore the results are not applicable to the lactoferrin content in pasteurized milk.

Paulsson et al. (1993) looked at the thermal behavior of HSTS pasteurized and UP-treated bovine lactoferrin and iron-saturated lactoferrin. Pasteurized lactoferrin showed some signs of denaturation but iron-saturated lactoferrin was not affected by pasteurization. The UP-treatment denatured both lactoferrin and iron-saturated lactoferrin. The thermal denaturation (protein unfolding) is reversible upon cooling (Ruegg et al. 1977). Although pasteurization does not denature lactoferrin, its affect on the biological properties of the protein has yet to be determined.

TABLE 10
Concentration of Lactoferrin (mg/ml) in Cow's Colostrum and Milk

Cow's Colostrum mg/ml	Cow's Milk mg/ml	Reference
	0.1	Barth and Behnke 1997
	Traces	Gordon and Kalan 1974
	0.02-0.2	Masson and Heremans 1971
2-5	0.02-0.2	Reiter 1978
	Traces	Rudloff and Kunz 1997
	0.02-0.2	Tomita 1999
2-5	0.1-0.3	Welty et al. 1975

Using food intake data from the 1994-96 United States Department of Agriculture's (USDA) Continuing Survey of Food Intakes by Individuals (CSFII), ENVIRON determined the average intake of milk and milk products on both a gram per day (g/d) and gram per kilogram of body weight per day (g/kg bw/d) basis (Table 11). The CSFII 1994-96 is a two nonconsecutive day database of dietary information from individuals of all ages that was collected between January 1994 and January 1997 through in-person interviews using 24-hour recalls. The CSFII 1994-96 sample was a stratified, multistage area probability sample. Sampling weights, provided by USDA, compensate for variable probabilities of selection, differential response rates, and possible deficiencies in the sampling frame. Weighted data were used to calculate mean intakes and 90th percentiles that are listed in the Table 11. The survey database also includes data

on nutrients, like protein, for each food reported. Unless otherwise noted, the discussion of milk consumption in this section refers to milk as well as milk products that are listed in Appendix D.

On average, children 1 to12 and teens 13 to 19 years old consume approximately 396 and 377 g milk/day, respectively. This is equivalent to 38 to 40 mg lactoferrin/day. Adults (20+) consume less milk, 240 g/d; thus their intake of lactoferrin is equal to about 24 mg/day. Consumption of lactoferrin for milk consumers in the 90th percentile averages 73 mg/d for children, 75 mg/d for teens and 50 mg/d for adults.

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TABLE 11
Estimated Daily Intakes of Lactoferrin from Milk and Milk Products[1]

Population Group	Dairy Products		Protein in Dairy Products		Lactoferrin in Dairy Products	
	2 Day Average Intake per User	90th Percentile 2 Day Average Intake per User	2 Day Average Intake per User	90th Percentile 2 Day Average Intake per User	2 Day Average Intake per User[2]	90th Percentile 2 Day Average Intake per User
Children (1-12 yr.)	395.6 g/d (18.8 g/kg bw/d)	731.1 g/d (37.6 g/kg bw/d)	13.1 g/d (0.6 g/kg bw/d)	24.2 g/d (1.248 g/kg bw/d)	40 mg/d (1.9 mg/kg bw/d)	73 mg/d (3.8 mg/kg bw/d)
Teens (13-19 yr.)	377.1 g/d (6.2 g/kg bw/d)	746.8 g/d (12.4 g/kg bw/d)	12.5 g/d (0.2 g/kg bw/d)	25.1 g/d (0.417 g/kg bw/d)	38 mg/d (0.6 mg/kg bw/d)	75 mg/d (1.2 mg/kg bw/d)
Adults (20 yr. +)	240.3 g/d (3.3 g/kg bw/d)	500.4 g/d (7.1 g/kg bw/d)	8.0 g/d (0.1 g/kg bw/d)	16.9 g/d (0.238 g/kg bw/d)	24 mg/d (0.3 mg/kg bw/d)	50 mg/d (.7 mg/kg bw/d)

[1]Estimates reflect consumption of milk and milk products which includes all fluid and flavored milks, reconstituted milk, milkshakes, ice cream, puddings, cream and whipped cream.
Analyses include individuals who provided dietary recalls for two days and reported a body weight.
With the exception of the number of users, all results are based on weighted data; estimates calculated with WesVar Complex Samples 3.0 (SPSS, 1998).
Data source: 1994-96 Continuing Survey of Food Intakes by Individuals
[2] Calculations based on 0.1 mg lactoferrin per g of milk or 0.3% of protein consumption (Barth and Behnke 1997).

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b) Infant Formula

(1) U.S. Infant Formula

Breast milk is generally recognized as the standard for good infant nutrition. Thus many infant formulas are patterned after breast milk to provide the right quality and balance of protein, fat, and carbohydrate for the first 0 to 12 months of life.

Infant formula intakes were estimated using data from the USDA Continuing Survey of Food Intake (CSFII 1994-96). Infants 1 to 4 months and 5 to 11 months of age consumed approximately 1,031 ml (194 ml/kg bw/d) and 814 ml (97 ml/kg bw/d) infant formula/day, respectively. Average body weights of infants 1 to 4 and 5 to11 months were 5.3 kg and 8.4 kg, respectively.

The protein composition of infant formula varies from manufacturer to manufacturer. For example, the protein in Similac (Ross Labs) is composed of approximately 48% whey protein, while Enfamil (Mead Johnson) and Good Start (Nestle Carnation) contain 60 and 100% whey protein, respectively. Since lactoferrin accounts for approximately 1.4% of whey protein (Barth and Behnke 1997) the amount of lactoferrin available from infant formula ranges between 102 to 238 μg/ml (Table 12).

Using intake estimates and lactoferrin concentrations, infants 1 to 4 months consume between 105 to 245 mg lactoferrin/day (20 to 46 mg/kg/day). Lactoferrin intake by infants 5 to 11 months ranges from 83 to 194 mg/day (10 to 23 mg/kg/day).

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TABLE 12
Daily Intake of Lactoferrin from Infant Formula

Product	Manufacturer, Country	Protein Concentration (As consumed)	Whey Protein Concentration	Lactoferrin Concentration [4]	Total Daily Intake of Lactoferrin [5] (1-4 months)	Total Daily Intake of Lactoferrin [6] (5-11 months)
Enfamil	Mead Johnson	2.1 g/141 ml (14.9 mg/ml)	8.94 mg/ml [1]	125.2 µg/ml	129 mg/day (24.3 mg/kg/day)	102 mg/day (12 mg/kg/day)
Good Start	Nestle Carnation	2.4 g/141 ml (17.0 mg/ml)	17.0 mg/ml [2]	238 µg/ml	245 mg/day (46 mg/kg/day)	194 mg/day (23 mg/kg/day)
Similac	Ross Labs	2.19 g/141 ml (15.2 mg/ml)	7.3 mg/ml [3]	102.2 µg/ml	105 mg/day (19.8 mg/kg/day)	83 mg/day (9.9 mg/kg/day)

[1] Protein concentration is approximately 60% whey protein according to product advertising.
[2] Protein concentration is approximately 100% whey protein according to product advertising.
[3] Protein concentration is approximately 48% whey protein according to product advertising.
[4] Lactoferrin content is equivalent to 1.4% of whey protein concentration (Barth and Behnke 1997).
[5] Calculation based on children (0-4 months of age, with an average body weight of 5.3 kg) consuming 1,031 ml of infant formula per day (194 ml/kg bw/day) as reported in the CSFII 1994-96 Survey.
[6] Calculation based on children (5-11 months of age, with an average body weight of 8.4 kg) consuming 814 ml of infant formula per day (97 ml/kg bw/day) as reported in the CSFII 1994-96 Survey.

(2) Supplemented Infant Formula

Several European companies enrich infant formula with bovine lactoferrin. Concentrations and intake of supplemented lactoferrin are shown in Table 13. Infants 1 to 4 months old consume an additional 66 to 111 mg/d (12 to 21 mg/kg bw/d) of lactoferrin while 5 to 11 month olds get an additional 52 to 88 mg/d (3 to 10 mg/kg bw/d). These infant formulas which have been enriched with lactoferrin also contain lactoferrin as a component of the whey protein. Thus total daily intake of lactoferrin would be equivalent to supplemented levels plus the whey protein-derived levels. Sufficient information to calculate lactoferrin concentration from the whey protein was not available for these formulas. However, based on lactoferrin consumption from U.S. infant formulas (Table 12), infants 1 to 4 months would consume a total of 171 to 356 mg/d (32 to 67 mg/kg bw/d) of lactoferrin and infants 5 to 11 months would consume 135 to 282 mg/d or 13 to 33 mg/kg bw/d.

Morinaga Milk Industry Co., Ltd. first introduced lactoferrin enriched infant formula to the Japanese market in 1986. Dry Milk Hagukumi and the follow-up formula, Chil-Mil AYUMI both contain lactoferrin, 50 mg and 45 mg/100g, respectively. Since their release, Morinaga has sold approximately 44,600 tons of Chil-Mil AYUMI and 57,500 tons of Dry Milk Hagukumi. Morinaga maintains a Consumer Service Department so customers can call to ask questions and/or register complaints about their products. Since April 1997, the company has recorded 11,508 calls concerning Dry Milk Hagukumi and 4,476 calls about Chil-Mil AYUMI. To date, the manufacturer states that none of the calls have been associated with the lactoferrin contained in either product (Hayasawa, 1999)

TABLE 13
Intake of Supplemental Lactoferrin from Enriched Infant Formulas

Product	Manufacturer, Country	Supplemented Lactoferrin Concentration [1]	Product Reconstitution	Supplemented Lactoferrin Concentration "As consumed"	Intake of Supplemental Lactoferrin [2,4] (1-4 months)	Intake of Supplemental Lactoferrin [3,4] (5-11 months)
Chil-Mil AYUMI	Morinaga, Japan	45 mg/100 g powder	14.0 g/90 ml H_2O	70 μg/ml	72 mg/day (14 mg/kg/day)	57 mg/day (7 mg/kg/day)
Dry Milk Hagukumi	Morinaga, Japan	50 mg/100 g powder	13.0 g/91 ml H_2O	71 μg/ml	73 mg/day (14 mg/kg/day)	58 mg/day (7 mg/kg/day)
Imperial No. 1	Namyang Dairy Prdts. Co., Korea	80 mg/100 g powder	13.0 g/100 ml H_2O	104 μg/ml	107 mg/day (20 mg/kg/day)	85 mg/day (10 mg/kg/day)
Mama Omega I	Maeil Dairy, South-Korea	75 mg/100 g powder	13.0 g/90 ml H_2O	108 μg/ml	111 mg/day (21 mg/kg/day)	88 mg/day (10 mg/kg/day)
Regent No. 1	Namyang Dairy Prdts. Co., Korea	75 mg/100 g powder	13.0 g/100 ml H_2O	97 μg/ml	100 mg/day (19 mg/kg/day)	79 mg/day (9 mg/kg/day)
Vitalac 1 (0-6 months)	PT Sari Husada, Indonesia	45 mg/100 g powder	12.7 g/90 ml H_2O	64 μg/ml	66 mg/day (12 mg/kg/day)	52 mg/day (3 mg/kg/day)
Vitalac 2 (6-24 months)	PT Sari Husada, Indonesia	45 mg/100 g powder	14.1 g/90 ml H_2O	71 μg/ml	73 mg/day (14 mg/kg/day)	58 mg/day (4 mg/kg/day)

[1] Values from product label.
[2] Calculation based on children (0-4 months of age, with an average body weight of 5.3 kg) consuming 1,031 ml of infant formula per day (194 ml/kg bw/day) as reported in the CSFII 1994-96 Survey.
[3] Calculation based on children (5-11 months of age, with an average body weight of 8.4 kg) consuming 814 ml of infant formula per day (97 ml/kg bw/day) as reported in the CSFII 1994-96 Survey.
[4] These infant formulas are enriched with lactoferrin but they also contain lactoferrin as a component of the whey protein. Sufficient information to calculate lactoferrin concentration from whey protein is not available for these formulas. However, based on calculations derived from U.S. infant formulas, lactoferrin intakes from the whey protein component of the formula range from 105-245 mg/d for infants 1-4 months and 83-194 mg/d for infants' 5-11 months. Therefore total daily intake of lactoferrin is equivalent to supplemented levels plus the whey protein derived levels.

c) Dietary Supplements

Lactoferrin is also currently marketed in dietary supplement form (products listed in Table 14) with dose recommendations ranging from 10 to 1200 mg/day.

TABLE 14
Concentration of Lactoferrin in Dietary Supplements[1]

Product	Manufacturer, Country	Lactoferrin Concentration	Recommended Dosage	Total Daily Intake
Intestinal Flora Factors	Ethical Nutrients, USA	NR	8-12 tablets/day	
Jarro-Dophilus + Lactoferrin	Jarrow Formulas, Canada	200 mg/capsule	1-3 capsules/day	200-600 mg
Lactoferrin	GNC Corp., USA	50 mg/tablet	1-2 tablets/day	50 mg
Lactoferrin	Jarrow Formulas, Canada	250 mg/capsule	1 capsule/day	250 mg
Nutri Elle	Tonipharm Labs., France	10 mg/ capsule	1 capsule/day	10 mg
Nutri Femme	Tonipharm Labs., France	20 mg/capsule	1 capsule/day	20 mg
Nutrinatal	Tonipharm Labs., France	50 mg/capsule	1 capsule/day	50 mg
Oenobiol	Oenobiol Labs., France	356 mg/ tablet	NR	
Prime Colostrum, Lactoferrin, Pectin	Lactoferrin Products Co., USA	10 mg/lozenger	1 lozenger/day	10 mg
Sentosa Fe	Sentosa, Taiwan	100 mg/tablet	1-2 tablets/day	100-200 mg
Ultra Immune	Lactoferrin Inc. USA	50 mg/tablet	1 tablet/day	50 mg
Uzer Ferrine	Pharmafood, The Netherlands	100 mg/tablet	6-12 capsules/day	600-1200 mg

[1] Data retrieved from the World Wide Web.

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B. Estimated Daily Intake (EDI) of Bovine lactoferrin from Proposed Use

Bovine lactoferrin is proposed for use as an ingredient in sports and functional foods (proposed substitution by weight of 0.25% for wet/liquid products and 0.025% for dry/powdered products), and infant formulas (proposed substitution by weight of 0.1%).

The EDI of lactoferrin from sports and functional foods is presented in Table 15. The proposed EDI of bovine lactoferrin through its use in sports and functional foods is 1.0 and 2.1 g/day at the mean and 90th percentile of consumption. (The consumption of these foods is intended to supplement normal dietary intake of up to 75 mg/day from dairy sources, resulting in a total mean daily intake of up to 1.1 g/day.) A total daily intake of lactoferrin using consumption values from all products combined was not calculated because data showed that the user groups for these products were virtually independent. Therefore, an individual will be expected to consume only one type of product on any given day.

The proposed EDI of bovine lactoferrin through its use in infant formula is presented in Table 16. The proposed EDI for infants 0 to 4 months is 1.0 and 1.4 g/day at the mean and 90th percentile of consumption; the proposed EDI for infants 5 to 11 months is 0.81 and 1.25 g/day at the mean and 90th percentile of consumption. (The consumption of supplemental lactoferrin from formula is in addition to the lactoferrin available from the whey protein which can result in consumption of up to 245 mg/day. Therefore, total mean daily intake of lactoferrin from infant formulas would be up to 1.3 g/day. Because infant formula is used as a sole source of nutrition in infants up to 4 months of age and cow's milk is not recommended for infants under 1 year of age, infant formula will be considered to be the only source of lactoferrin ingestion in non-breast-fed infants under 1 year.)

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TABLE 15
Estimated Daily Intake of Lactoferrin from Sports and Functional Foods Enriched with Lactoferrin [1]

Population Group	2 Day Average Intake per User (g per day)		90th Percentile 2 Day Average Intake per User (g per day)		2 Day Average Intake per User (g/kg bw/day)		90th Percentile 2 Day Average Intake per User (g/kg bw/day)	
MEAL REPLACEMENTS[2]	Food	Lactoferrin[3]	Food	Lactoferrin	Food	Lactoferrin	Food	Lactoferrin
Children (1 to 12 years)								
dry meal replacements	25.2	0.006	36.0	0.009	1.111	< 0.001	1.097	< 0.001
fluid meal replacements	207.8	0.520	356.0	0.890	8.815	0.022	18.565	0.046
solid meal replacements	22.9	0.057	41.8	0.105	0.948	0.002	1.657	0.004
Teens (13 to 19 years)								
dry meal replacements	48.6	0.012	62.0	0.016	0.618	< 0.001	0.770	< 0.001
fluid meal replacements	416.4	1.041	835.0	2.088	6.220	0.016	12.253	0.031
solid meal replacements	24.8	0.062	40.5	0.101	0.438	0.001	0.740	0.002
Adults (20 years +)								
dry meal replacements	36.9	0.009	73.5	0.018	0.495	< 0.001	0.968	< 0.001
fluid meal replacements	357.0	0.893	687.7	1.719	4.917	0.012	8.605	0.022
solid meal replacements	29.7	0.074	56.3	0.141	0.409	0.001	0.769	0.002

Notes:

1 Analyses include individuals who provided dietary recalls for two days and reported a bodyweight; Results are based on weighted data; estimates calculated with WesVar PC Version 2.12
Data source: 1994-96 Continuing Survey of Food Intakes by Individuals

2 Estimates reflect consumption of meal replacements and "diet" type beverages, powders and supplements; granola, breakfast and high energy sports bars; and sport beverages: "dry" foods include powders and unreconstituted mixes, "fluid" foods include liquids and reconstituted mixes, "solid" foods include bars

3 Lactoferrin intake represents combined intake from all food sources (0.0025 g lactoferrin per gram fluid and solid meal replacements and 0.00025 g lactoferrin per gram dry meal replacements)

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TABLE 16
Estimated Daily Intake of Supplemental Lactoferrin from Infant Formula Enriched with Lactoferrin[1]

	Infant Formula		Supplemental Lactoferrin		Total Lactoferrin Intake[2]	
Infants	**Mean Daily Intake per user**	**90th percentile of Intake per user**	**Mean Daily Intake per user**	**90th percentile of Intake per user**	**Mean Daily Intake per user**	**90th percentile of Intake per user**
Infants 0 to 4 months	1031 g/d (194 g/kg bw/d)	1403 g/d 262 g/kg bw/d	1.03 g/d 0.19 g/kg bw/d	1.40 g/d 0.26 g/kg bw/d	1.1-1.3 g/d 0.21-0.24 g/kg bw/d	1.5-1.6 g/d 0.28-0.31 g/kg bw/d
Infants 5 to 11 months	814 g/d (97 g/kg bw/d)	1254 145 g/kg bw/d	0.81 g/d 0.10 g/kg bw/d	1.25 g/d 0.15 g/kg bw/d	0.9-1.0 g/d 0.11-0.12 g/kg bw/d	1.3-1.4 g/d 0.16-0.17 g/kg bw/d

Notes:

[1] Intake estimates reflect the daily average intake consumption of infant formula based on intake reported during two days.
Breast feeding infants were excluded from analyses.
Data source: USDA 1994-96 Continuing Survey of Food Intakes by Individuals; weighted data.
Statistics calculated with WesVar PC Version 2.12

[2] In addition to 0.1% supplementation of lactoferrin, infant formula also contains lactoferrin as a component of the whey protein. Based on calculations derived from U.S. infant formulas, lactoferrin intake ranges from 0.105-0.245 g/d (0.02- 0.05g/kg bw/d) for infants 1-4 months of age and 0.083-0.194 g/d (0.01-0.02 g/kg bw/d) for infants 5-11months old. Therefore, total lactoferrin intake is equivalent to proposed supplemental levels plus lactoferrin concentration from the whey protein.

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IV. INTENTED EFFECTS

A. Lactoferrin and Modulation of the Immune Response

Inflammation is fundamentally a protective response to cell injury. When excessive in magnitude or duration, however, the otherwise beneficial effects of inflammation may be deleterious, impacting negatively on the recovery or healing of the host (Kruzel et al. 1998). Lactoferrin has been shown in several experimental models, both *in vitro* and *in vivo*, to modulate inflammation. These studies are reviewed in Table 17.

The outcomes of acute inflammation and the modulatory effect of lactoferrin can be summarized as follows (Kruzel et al. 1998): 1) acute inflammation, defined by infection, toxic mediators lipopolysaccharides (LPS) or trauma, leads to activation of the monocyte/macrophage system and stimulates the production of IL-1β, TNFα and GM-CSF; 2) this in turn activates circulating neutrophils; 3) and stimulates the production of fresh neutrophils and monocyte/macrophages from the bone marrow; 4) activated neutrophils degranulate at the site of injury and release lactoferrin; 5) by binding to the specific receptor on monocytes (Lönnerdal and Iyer 1995), lactoferrin reduces the production of IL-1β, and GM-CSF, which decreases the production of fresh monocyte/macrophages and neutrophils (Mattsby-Baltzer et al. 1996). In addition, lactoferrin released from neutrophils can bind LPS, reducing its ability to prime neutrophils (Wang et al. 1995; Cohen et al. 1992). Although generally beneficial to the host, inflammatory processes are potentially destructive to surrounding tissues. The inflammatory response can spread from the local environment and induce generalized systemic response that may become self-perpetuating by overproduction of pro-inflammatory cytokines. By blocking the deleterious effects of those cytokines, lactoferrin has the potential to control the development of systemic inflammation (Kruzel et al. 1998).

The ability of bovine lactoferrin pretreatment to protect against lethal shock induced by iv administration of endotoxin was demonstrated in a germfree, colostrum-deprived, immuologically virgin piglet model (Lee et al. 1998). The mechanism of the lactoferrin protection was determined to be inhibition of LPS binding to monocytes/macrophages through lactoferrin binding activity for the lipid A portion of LPS. This, in turn, prevented induction of monocyte/macrophage-derived inflammatory toxic cytokines.

Both human and bovine lactoferrin, as well as a pepsin-derived fragment of bovine lactoferrin (lactoferricin B) were found *in vitro* to suppress the IL-6 response in a monocytic cell line when stimulated by LPS (Mattsby-Baltzer et al. 1996). Thus, the anti-inflammatory action of both the human and bovine lactoferrin appears to be through suppressive effects on cytokine release.

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Nakajima et al. (1999) investigated the effect of orally administered lactoferrin on cytokine production of spleen cells from BALB/c mice. Spleen cells were cultured with concanavalin A 1 to 4 days after oral administration of bovine lactoferrin. Results indicated that enterally administered lactoferrin affects the host immune responses by modulating cytokine responses. The immune modulation induced by lactoferrin has been hypothesized to be a potential mechanism underlying its inhibition of tumor development in several animal models (Ushida et al. 1998, 1999; Iigo et al. 1999; Sekine et al. 1997a,b).

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TABLE 17 Biological Functions Of Lactoferrin Immunomodulatory Effect			
Study Design	**Results**	**Comments**	**Reference**
The ability of bovine lactoferrin to protect against lethal shock induced by intravenously administered endotoxin was studied in the germfree, colostrums-deprived, immunologically "virgin" piglet model. Piglets were fed lactoferrin or bovine serum albumin (BSA) prior to challenge with iv E. coli lipopolysaccharide. Temperature, clinical symptoms, and mortality were tracked for 48 hours following lipopolysaccharide administration.	Prefeeding with lactoferrin resulted in a significant decrease in piglet mortality compared to feeding BSA. Protection by the lactoferrin was also correlated with both resistance to induction of hypothermia by endotoxin and an overall increase in wellness as quantified by a toxicity score developed for these studies. In vitro studies showed that lipopolysaccharide binding to monocytes was inhibited by lactoferrin in a dose-dependent fashion.	Death from septic shock is thought to be a consequence of the effect of monocyte-derived cytokines such as tumor necrosis factor alpha, interleukin-1 and interleukin-6, which are induced in response to bacterial endotoxin (lipopolysaccharide). Lactoferrin has been shown *in vitro* to be bacteriostatic and bacteriocidal. Two possible mechanisms have been proposed: one is to chelate iron ions which are essential for bacterial growth, and the other is to destabilize the outer membrane of gram-negative organisms. In this study, it was demonstrated that the mechanism of lactoferrin action *in vivo* may be inhibition of lipopolysaccharide binding to monocytes/ macrophages through lactoferrin binding activity for the lipid A portion of lipopolysaccharide. This, in turn, prevents induction of monocyte/ macrophage-derived inflammatory-toxic cytokines.	Lee et al. 1998
The purpose of this *in vitro* study was to characterize lipopolysaccharide inactivation by neutrophils and identify the factor responsible for inactivation.	A single lipopolysaccharide inactivating factor was purified from medium by heparin-agarose chromatography. SDS-PAGE showed a single band at 80 kDa, which was identified as lactoferrin by immunoblotting. Antilactoferrin immunoglobulin G removed the lipopolysaccharide inactivating activity from purified lactoferrin and cell-free medium. Lactoferrin inactivation of lipopolysaccharide was restricted to neutrophils; monocytes do not secrete lactoferrin and they did not inactivate lipopolysaccharide.	After neutrophils are primed by lipopolysaccharide from bacteria or by tumor necrosis factor alpha or interleukin-8 from monocytes, the neutrophils produce abundant O_2^- when triggered by bacterial chemotactic factors. Lipopolysaccharide, the glycolipid component on the outer surface of the outer membrane of gram-negative bacteria, is the premier priming agent for neutrophils and one of the most important mediators of inflammation. The highly conserved lipid A region of lipopolysaccharide is responsible for most	Wang et al. 1995

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TABLE 17 Biological Functions Of Lactoferrin Immunomodulatory Effect			
Study Design	**Results**	**Comments**	**Reference**
		of the biologic activities of lipopolysaccharide. Lactoferrin secreted by neutrophils is responsible for the inactivation of lipopolysaccharide. The unique ability of neutrophils to inactivate lipopolysaccharide implies an important role of neutrophils and lactoferrin in the regulation of inflammation induced by gram-negative bacteria.	
The purpose of this *in vitro* study was to investigate whether or not human milk lactoferrin in comparison with bovine lactoferrin can affect the IL-6 release from human monocytic cells. Lactoferrin was added to fresh human monocytes or cultured monocytic cells.	Human, as well as bovine, lactoferrin, and a bactericidal pepsin-derived fragment of bovine lactoferrin (lactoferricin B), were found to suppress the IL-6 response in a monocytic cell line when stimulated by lipopolysaccharide. The suppression of bovine lactoferrin was similar to or higher than that of human lactoferrin. Results suggest an anti-inflammatory activity of both human and bovine lactoferrin and of the lactoferrin fragment, through their suppressive effects on the cytokine release.	Lactoferrin is associated with host defense at the mucosal surfaces through its antibacterial and iron-binding properties. Recently, a pepsin-cleaved fragment of human and bovine lactoferrin, lactoferricin B, was found to contain the structural domain responsible for the bactericidal properties of lactoferrin. After binding of bacterial lipopolysaccharide to macrophages, T cells and cultured human monocytes, these cells synthesize TNF-α, IL-1, IL-6 and colony-stimulating factor. Cells participating in the inflammatory response carry several different lipopolysaccharide binding receptors. Such cells also have receptors for lactoferrin. This study has shown that lactoferrin exerts an inhibitory effect on the production of the lipopolysaccharide induced IL-6 response when added to fresh human monocytes or cultured monocytic cells. Thus, lactoferrin may act as a potential anti-inflammatory agent.	Mattsby-Baltzer et al. 1996

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TABLE 17 Biological Functions Of Lactoferrin Immunomodulatory Effect			
Study Design	**Results**	**Comments**	**Reference**
The purpose of this *in vitro* study was to investigate the potential physiologic consequences of the interaction between lactoferrin and lipopolysaccharide, including the ability of lipopolysaccharide to induce a priming response in human neutrophils.	Lactoferrin prevents hydroxyl radical formation by binding iron, even at low pH. Lactoferrin inhibited iron-catalyzed formation of hydroxyl radical in the presence of lipopolysaccharide at pH 7.4 and 4.5. Lactoferrin also inhibited lipopolysaccharide priming of neutrophils. Inhibition of lipopolysaccharide priming was observed whether apolactoferrin or iron-saturated lactoferrin was used.	These results show that lipopolysaccharide should not be expected to affect the free radical biology of lactoferrin, which is a crucial physiologic function of this protein. However, lactoferrin inhibits lipopolysaccharide priming, and this requires consideration in experimental models of inflammation. It appears that *in vitro*, lactoferrin can form a complex with lipopolysaccharide, which decreases its ability to prime neutrophils.	Cohen et al. 1992
The purpose of this study was to investigate the effect of human lactoferrin on humoral response to sheep red blood cells *in vivo* and *in vitro*.	Human milk lactoferrin at a concentration ranging from 1 to 10 μg/ml stimulated up to 5 times the humoral immune response to sheep red blood cells, expressed as the number of plaque-forming cells, when injected into mice 3 hours before immunization. Further, lactoferrin-treated thymocytes given iv in mice enhanced the immune response to sheep red blood cells to the same extent as IL-1. In vitro, studies showed that $CD4^-$ $CD8^-$ thymocytes incubated with lactoferrin and added to the splenocyte cultures, increased the immune response to sheep red blood cells. After an overnight incubation with lactoferrin, $CD4^-$ $CD8^-$ thymocytes acquired the CD4 antigen characteristic for the helper cell phenotype.	Results suggest that lactoferrin stimulates the immune response by a process which involves the promotion of T cell differentiation.	Zimecki et al. 1991
The effect of orally administered lactoferrin on cytokine production of spleen cells was investigated. Spleen cells from BALB/c mice were cultured with concanavalin A 1 to 4 days after the oral administration of bovine lactoferrin.	Concentrations of IFN-γ in the supernatants were enhanced by lactoferrin feeding, while those of IL-4 were not. When stimulated by anti-CD-3 antibody, IFN-γ production by CD4+ T cells fractionated from spleen cells was augmented by the oral administration of lactoferrin. On the other hand, in response to lipopolysaccharide, spleen cells from the mice fed lactoferrin secreted enhanced levels of IL-10. Levels of IFN-γ secretion from the spleen cells were not affected. While IL-10 production in response to	Studies reported in the literature have suggested that lactoferrin has a preventive effect on tumor development in rats, helps in the cure of dermatophytosis in guinea pigs and improves stomatitis in cats when administered orally. Although the mechanisms for the resistance to these diseases have not been clearly defined, the biological effects of orally administered lactoferrin on the host immune system have	Nakajima et al. 1999

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TABLE 17 Biological Functions Of Lactoferrin Immunomodulatory Effect			
Study Design	**Results**	**Comments**	**Reference**
	lipopolysaccharide by CD11c+ cells from spleen cells was promoted by lactoferrin feeding, the cytokine secretion from CD11b+ cells was not affected.	been suggested. Selective cytokine production and the resultant immune response may play a protective or pathogenic role in various pathological conditions. IFN-γ has been shown to promote anti-viral activity, anti-microbial activity, and anti-tumor activity by a variety of immunomodulatory functions. Results indicate that enterally administered lactoferrin affects the host immune responses by modulating cytokine responses and suggest the immune modulatory effect of lactoferrin.	
The purpose of the study was to investigate the effects of oral administration of bovine lactoferrin on intractable stomatitis in feline immunodeficiency virus (FIV)-positive and FIV-negative cats, and phagocytosis of neutrophils in healthy and ill cats, simultaneously. Seven ill cats with diagnosis of intractable stomatitis (4 FIV positive and 3 FIV negative) and 7 healthy FIV-negative cats were used. Lactoferrin (40 mg/kg) was applied topically to the oral mucosa for 14 days.	Treatment with lactoferrin improved clinical signs of the disease (pain-related response, salivation, appetite, and oral inflammation). Phagocytic activity of neutrophils increased after lactoferrin treatment. This effect was observed in healthy and ill cats.	Lactoferrin improved intractable stomatitis and concurrently enhanced the host defense system.	Sato et al. 1996

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TABLE 17 Biological Functions Of Lactoferrin Immunomodulatory Effect			
Study Design	**Results**	**Comments**	**Reference**
The purpose of this study was to investigate the effect of lactoferrin on the processes leading to inflammation that could affect intestinal polyp development in the Apc^{Min} mouse model. (These mice demonstrate spontaneous development of numerous intestinal polyps due to a germ-line mutation in one allele of the murine Apc gene, with tumors arising through loss of heterozygosity. It is similar to an inherited form of human intestinal cancer, familial adenomatous polyposis). Mice received either no dietary supplement, 0.2% bovine lactoferrin or 2% bovine lactoferrin, respectively, beginning at 6 weeks of age and were sacrificed after 8 weeks.	There was an overall tendency for a reduction in the total number of polyps in the small intestine in the animals treated with bovine lactoferrin, along with a significant suppression in the jejunum at the 2% dose. In addition, body growth suppression, presumed to be due to anemia and/or intussusception as a consequence of numerous polyps in the intestine, was alleviated. No toxic effects were observed in the intestinal epithelium.	Results demonstrate that bovine lactoferrin can inhibit the spontaneous intestinal tumorigenesis that occurs in the Apc^{Min} mouse model, without any evidence of toxicity. Because bovine lactoferrin reduced the number of relatively small polyps, its effects may be greatest on the initial stage of polyp development.	Ushida et al. 1998
Bovine lactoferrin, shown to be a strong chemopreventive of colon carcinoma development, was examined for its influence on other organs using a rat multi-organ carcinogenesis model. Male F344 rats, aged 6 weeks, were treated sequentially with diethylnitrosamine (DEN, ip), dihydroxy-di-N-propylnitrosamine (DHPN, drinking water) and N-nitrosomethyl-benzylamine (NMBA, s.c.) during the first 8 weeks and then bovine lactoferrin was administered in the basal diet, at a dose of 2, 0.2, 0.02 or 0.002%. All surviving animals were	There was a tendency for reduction in the development of papillomas in the esophagous of bovine lactoferrin treated animals, along with a significant suppression of relatively large-sized papillomas (more than 50 mm^3 volume) at the 0.2% dose. The multiplicity of tumors (adenomas and carcinomas) in the lung was also decreased in animals fed 0.02% bovine lactoferrin compared to the control group. No enhancing or inhibitory effects of bovine lactoferrin on tumor development in other organs were noted.	Although no dose-response was observed, bovine lactoferrin tended to inhibit esophagus and lung carcinogenesis at the 0.002 - 2% doses, along with a significant inhibition at the 0.2% dose in the esophagus and at the 0.02% dose in the lung. The question of whether this effect is due to lactoferrin stimulation of immune responses remains to be determined.	Ushida et al. 1999

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TABLE 17 Biological Functions Of Lactoferrin Immunomodulatory Effect			
Study Design	**Results**	**Comments**	**Reference**
killed at week 41.			
In order to determine the effects of lactoferrin on tumor growth and metastasis, bovine lactoferrin, as well as bovine lactoferrin hydrolysate and lactoferricin, active products generated by acid-pepsin hydrolysis, were administered orally to BALB/c mice bearing subcutaneous implants of the highly metastatic colon carcinoma 26 (Co 26Lu).	Bovine lactoferrin and the hydrolysate demonstrated significant inhibition of lung metastatic colony formation from sc implanted tumors without appreciable effects on tumor growth. On the other hand, bovine lactoferrin did not exert marked anti-metastatic activity in athymic nude mice bearing Co 26Lu, although it had a tendency to inhibit the lung metastatic colony formation associated with anti-asialoGM1 antibody treatment.	Bovine lactoferrin and its derivative demonstrated marked antimetastatic effect by the oral route.	Iigo et al. 1999
The influence of concomitant administration of bovine lactoferrin on induction of aberrant crypt foci by azoxymethane was investigated in male F344 rats. Groups of animals were given sc injections of 15 mg/kg azoxymethane and received dietary supplementation with 2% or 0.2% bovine lactoferrin or *B. longum*. Timing of the azoxymethane treatment varied.	Bovine lactoferrin (2%) and 3% *B. longum*, as a positive control, significantly decreased the numbers of aberrant crypt foci, as well as the total numbers of aberrant crypts. Most importantly, large size foci composed of four or more crypts were always significantly decreased by 2% bovine lactoferrin. Additional investigation of the natural killer activity of spleen cells demonstrated enhancement by bovine lactoferrin and *B. longum,* in line with the levels of influence on foci induction.	The results of the study provide evidence of an inhibitory potential of bovine lactoferrin against induction of aberrant crypt foci by azoxymethane when given in the initiation and early post-initiation stages. Aberrant crypt foci are generally accepted as being relevant endpoint lesions of colonic cancers. With regard to the mechanisms underlying the bovine lactoferrin inhibitory influence, results of studies suggest a role of lactoferrin's regulation of the immune response. The results from this study suggest a mechanistic role for the stimulation of NK cell activity.	Sekine et al. 1997a

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TABLE 17 Biological Functions Of Lactoferrin Immunomodulatory Effect			
Study Design	**Results**	**Comments**	**Reference**
The influence of bovine lactoferrin on colon carcinogenesis was investigated in male F344 rats treated with azoxymethane. Following three weekly injections of azoxymethane, animals received 2 or 0.2% bovine lactoferrin for 36 weeks.	No effects indicative of toxicity were noted, but significant reduction in both the incidence and number of adenocarcinomas of the large intestine was observed with both doses. Thus, the incidences of adenocarcinomas in the groups receiving 2% and 0.2% bovine lactoferrin were 15% and 25%, respectively, in contrast to the 57.5% in the group.	Results indicate that bovine lactoferrin may have an inhibitory effect on colon cancer.	Sekine et al. 1997b

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B. Lactoferrin and Antimicrobial Action

1. Mechanism of Action

The ability of lactoferrin to inhibit the growth of a wide variety of pathogenic microorganisms has been well documented (Kalfas et al. 1991; Naidu et al. 1991; Zagulski et al. 1989,1998; Bhimani et al. 1999). Both bovine and human lactoferrin appear to possess this property, as demonstrated by the enhanced survival rates of mice pretreated with either bovine or human lactoferrin prior to challenge with *E. coli* (Zagulski et al. 1989). Lactoferricin, a potent antimicrobial peptide produced by gastric cleavage of lactoferrin, was also found to enhance survival rates of mice challenged with *Toxoplasma gondii* infection (Isamida et al. 1998). A review of the studies relevant to elucidating the mechanism of lactoferrin's antimicrobial effect both *in vitro* and *in vivo*, are summarized in Table 18.

The antimicrobial spectrum of lactoferrin includes stasis, cidal, cationic, phagocytic, and colonization/decolonization effects on susceptible microorganisms. Investigators have hypothesized that lactoferrin would impede iron utilization and cause bacteriostasis as a result of its iron-sequestering properties (Lönnerdal and Iyer 1995; Iyer and Lönnerdal 1993). However, the fact that some strains are resistant or unaffected by lactoferrin implies that bacteriostasis is not simply the result of iron sequestration (Bhimani et al. 1999). The selective interaction of lactoferrin with the microbial surface seems to play an essential role in regulating many of the antimicrobial events (Naidu and Arnold 1995; Alugupalli et al. 1995). A key event in this process is the binding of lactoferrin to specific outer membrane pore-forming proteins (porins) (Naidu and Bidlack 1998; Naidu et al. 1992, 1993; Naidu and Arnold 1994). Both human and bovine lactoferrin have been shown to bind to these proteins (Kishore et al. 1991). The magnitude of the lactoferrin-microbe interation is directly related to the bacterial susceptibility to lactoferrin (Naidu et al. 1991). Certain strains show a resistance to lactoferrin effects and do not demonstrate lactoferrin binding (Naidu et al. 1993). Although most bacteria express porins, the resistance of these bacteria was attributed to the shielding of the porin accessibility by the carbohydrate O-antigentic chains of lipopolysaccharide (LPS) (Naidu et al. 1993; Tigyi et al. 1992; Erdei et al. 1994).

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TABLE 18 Biological Functions Of Lactoferrin Antimicrobial Action			
Study Design	**Results**	**Comments**	**Reference**
Human isolates of *Porphyromonas gingivalis*, *Prevotella intermedia* and *Prevotella melaninogenica* from diseased periodontal pockets were examined for interaction with human lactoferrin in a standardized ^{125}I-labeled protein binding assay.	Strains of *Porphyromonas gingivalis*, *Prevotella intermedia* and *Prevotella melaninogenica* interact with human lactoferrin in a standardized radioisotope-labeled protein binding assay. Nonspecific binding accounted for about 10, 75 and 60% of the total binding in P. *intermedia*, P. *melaninogenica* and P. *gingivalis*, respectively. Unlabeled human or bovine lactoferrin effectively displaced labeled human lactoferrin binding. All three species recognized both human and bovine lactoferrin to a comparable degree.	Lactoferrin is found in parotid saliva at about 10 μg/ml. As a secretory glycoprotein of polymorphonuclear leukocytes (PMN), a role of lactoferrin has been suggested in various host defense processes. Lactoferrin is bactericidal during exponential phase and bacteriostatic during the late stationary phase of bacterial growth. It may play an important role in host defense at the mucosal surfaces by causing deprivation of iron from bacteria. In addition to these properties, lactoferrin was shown in this study to bind to several strains of periodontis-associated bacteria. The antimicrobial effect of lactoferrin is related to its adsorption to the microbial surface. In such interactions, the metal-chelating property of lactoferrin enables damage to lipid bilayers in the outer cell membrane and the release of lipopolysaccharides.	Kalfas et al. 1991

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TABLE 18 Biological Functions Of Lactoferrin Antimicrobial Action			
Study Design	**Results**	**Comments**	**Reference**
A total of 489 *Staphylococcus aureus* isolates from various clinical sources was examined for the occurrence of ^{125}I human lactoferrin binding.	Human lactoferrin binding was common among *S. aureus* strains associated with furunculosis (94.3%), toxic shock syndrome (94.3%), endocarditis (83.3%) and septicemia (82.8%) and other (nasal, vaginal or ocular) infections (96.1%). Furuncolosis-associated (low-invasive or localized) isolates were high-to-moderate binders of lactoferrin. In contrast, endocarditis-associated (high-invasive or systemic) isolates demonstrated low binding and did not bind lactoferrin at the above threshold level. *S. aureus* recognized human or bovine lactoferrin.	*S. aureus* is associated with human illnesses ranging from localized superficial infections such as folliculitis to severe systemic diseases such as fatal endocarditis and toxic shock syndrome. This pathogen requires iron for growth. During the early stages of pathogenesis, staphylococci confront inflammatory cell defense, mainly the polymorphonuclear leukocytes at mucosal surfaces. Among antibacterial compounds secreted by the PMNs is lactoferrin, an iron-binding protein. Lactoferrin deprives the invading pathogen of iron and provides an important defense at the host mucosal surface.	Naidu et al. 1991
A single dose of bovine lactoferrin was given intravenously (iv) to CFW mice 24 hours prior to the iv injection of the *E. coli* lethal dose (LD_{100}).	Bovine lactoferrin, when given iv, strongly accelerated the clearance rate of *E. coli* from the blood as well as its killing rate in the liver, lungs, spleen and kidney. Lactoferrin was much less effective in accelerating clearance of bacteria from the circulation when injected into animals subcutaneously. Unlike the case of clearing bacteria from the blood, however, subcutaneously injected lactoferrin efficiently stimulated killing of bacteria in internal organs similar to the case for the iv injected protein.	The results demonstrate that the protection afforded by lactoferrin against *E. coli* is primarily killing of the organisms. There is a dramatic acceleration of the clearing rate of liver *E. coli* from the blood.	Zagulski et al. 1998
A single intravenous dose of bovine lactoferrin was given to mice 24 hours before a challenge with a lethal dose of *E. coli*.	About 70% of mice pretreated with lactoferrin survived the challenge. The survival rates in control mice treated with *E. coli* alone and pretreated with bovine serum albumin were 4 and 8%, respectively. Human lactoferrin had almost the same protective effect as bovine lactoferrin.	This study demonstrated a protective role of iv administered lactoferrin in a systemic, nonspecific defense in mice during experimental infection with *E. coli*.	Zagulski et al. 1989

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TABLE 18 Biological Functions Of Lactoferrin Antimicrobial Action			
Study Design	**Results**	**Comments**	**Reference**
Human and bovine lactoferrins were assessed *in vitro* and *in vivo* for their antibacterial effects on *Staphylococcus aureus*. Lactoferrin for feeding experiments was prepared by dissolving 2 grams of bovine lactoferrin in water or whole bovine milk and supplied to test animals ad libitum 1 day prior to infection. Animals were fed the experimental diet for a period of 14 days and sacrificed. Lactoferrin solutions were also prepared for injection and given to mice iv on day minus one. Mice were injected with *S. aureus* on day zero of the study and then sacrificed on day 14.	Lactoferrins showed weak *in vitro* antibacterial activity while Fe-saturated lactoferrins showed no activity. When lactoferrin was given to mice by iv injection, there was a 30-50% reduction in kidney infections and viable bacterial counts in the kidneys decreased 5-12 fold. Lactoferrins were effective when given 1 day prior to the bacterial challenge, after which there was no significant effect even at doses up to 5 mg. Apo- and Fe-saturated forms of human and bovine lactoferrins were all equally effective. Feeding mice with 2% bovine lactoferrin in drinking water also reduced the kidney infections by 40-60% and viable bacterial counts, 5-12 fold.	Antimicrobial action of lactoferrin has been attributed to the ability of lactoferrin to chelate Fe strongly, making this essential nutrient inaccessible to the invading pathogens. Evidence suggests, however, that the antimicrobial activity is more complex than simple Fe chelation. Lactoferrins have bactericidal activity that may involve direct interaction with the cell surface. Mechanisms of antibacterial effects may include blockade of bacterial carbohydrate metabolism, or destabilization of the bacterial cell surface. This study demonstrates the protective effects of lactoferrin feeding and injections *in vivo* against systemic kidney infections induced by *S. aureus* in mice.	Bhimani et al. 1999

TABLE 18 Biological Functions Of Lactoferrin Antimicrobial Action			
Study Design	**Results**	**Comments**	**Reference**
This study was aimed at elucidating the effect of lactoferrin-porin interaction on bacterial growth. For this purpose, the lactoferrin susceptibility patterns of different clinical isolates of *E. coli* with various magnitudes of lactoferrin binding capacity were examined. The effect of lactoferrin on bacterial growth was tested by measuring conductance changes in the cultivation media and was compared with the magnitude of ^{125}I labeled lactoferrin binding in 15 clinical isolates of *E. coli*. To illustrate the extent of lipopolysaccharide (LPS) involvement, various rough mutants of *S. typhimurium* with different lengths of polysaccharide moiety were tested for lactoferrin binding and susceptibility.	The binding property was inversely related to the change in bacterial metabolic rate and was directly related to the degree of bacteriostasis. The MIC is defined as the smallest amount of lactoferrin required to elicit a complete inhibition of bacterial growth at the time point when the metabolism of the control (bacteria grown without lactoferrin) reached stationary phase. The magnitude of lactoferrin-bacterium interaction showed no correlation with the MIC of lactoferrin. In certain strains, lactoferrin at supraoptimal levels reduced the bacteriostatic effect. Thus, the lactoferrin concentration in the growth media was critical for the antibacterial effect. The magnitude of lactoferrin binding to whole cells is inversely related to the length of the LPS polysaccharide moiety. Accordingly, the low-lactoferrin binding smooth parent was less susceptible to lactoferrin, and at corresponding conditions the growth of the LPS-deficient mutant was inhibited by 50%. Results of the study with *S. typhimurium* suggest that there is a correlation between lactoferrin binding to porins and the lactoferrin mediated antimicrobial effect.	Studies with clinical isolates of E. coli revealed that the concentration of lactoferrin in the growth media is a critical factor for the antibacterial effect. In addition, there is a correlation between lactoferrin binding to porins and the lactoferrin mediated antimicrobial effect. The polysaccharide moiety of lipopolysaccharides shielded porins from the lactoferrin interaction and concomitantly decreased the antibacterial effect.	Naidu et al. 1993

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TABLE 18 **Biological Functions Of Lactoferrin** **Antimicrobial Action**			
Study Design	**Results**	**Comments**	**Reference**
The ability of *Shigella flexneri* to interact with lactoferrin was examined with a ^{125}I-labeled protein binding assay.	The percent binding of human lactoferrin and bovine lactoferrin to 45 *S. flexneri* strains was 19 ± 3 and 21 ± 3, respectively. ^{125}I-labeled human and bovine lactoferrin binding to strain M90T reached an equilibrium within 2 hours. Unlabeled human and bovine lactoferrin displaced the ^{125}I-labeled human lactoferrin-bacteria interaction in a dose-dependent manner. The cell envelope and outer membrane preparations showed three distinct horseradish peroxidase-lactoferrin reactive bands of about 39, 22, and 16 kDa. The 39 kDa component was also reactive to a monoclonal antibody specific for porin proteins of members of the family *Enterobacteriaceae*. The lactoferrin binding protein pattern was similar with bovine or human lactoferrin.	The results of this study establish that lactoferrin binds to specific components in the bacterial outer membrane; the heat-modifiable, anti-porin-reactive, and lipopolysaccharide-associated properties suggest that the lactoferrin binding proteins are porins in *S. flexneri*.	Tigyi et al. 1992
The purpose of this study was to examine the presence of a specific human lactoferrin binding protein in *S. aureus* and to characterize the lactoferrin-staphylococcal interaction in *S. aureus* strain MAS-89 (associated with toxic shock syndrome). A ^{125}I human lactoferrin binding assay was used.	The binding of ^{125}I human lactoferrin to strain MAS-89 reached saturation in <90 minutes and was maximal between pH 4 and 9. Unlabelled human lactoferrin displaced ^{125}I human lactoferrin binding. A Scatchard plot was non-linear; this implied a low affinity and high affinity binding mechanism. The staphylococcal lactoferrin binding protein was partially susceptible to proteolytic enzymes or periodate treatment and was resistant to glycosidases. An active binding protein with an apparent M_r of c. 450 Kda was isolated from strain MAS-89 cell lysate by ion-exchange chromatography on Q-sepharose. In SDS-PAGE, the reduced binding protein was resolved into two components of 67 and 62 Kda. The two components demonstrated a positive reaction with human lactoferrin-horseradish peroxidase in a Western blot.	The data from this study establishes that there is a specific receptor for human lactoferrin in *S. aureus*. This receptor-ligand interaction is distinct from the staphylococcal receptors binding to plasma or connective tissue proteins described previously.	Naidu et al. 1992

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TABLE 18 Biological Functions Of Lactoferrin Antimicrobial Action			
Study Design	**Results**	**Comments**	**Reference**
The purpose of this study was to elucidate the interaction between lactoferrin and binding sites on *E. coli* using ^{125}I-labeled human lactoferrin.	A band with a mass of approximately 37 kDa, which was reactive with horseradish peroxidase-labeled Lf, was identified in the boiled cell envelope and outer membrane preparations of a lactoferrin binding *E. coli* strain (E34663) and a non-lactoferrin binding strain (HH45), by SDS-PAGE and Western blotting. Such a band was not detected in the unboiled native cell envelope and outer membrane preparations. The molecular mass and the property of heat modifiability suggested that the lactoferrin-binding proteins were porins. OmpF and OmpC were identified as the lactoferrin binding proteins by urea-SDS-PAGE and Western blotting and by ^{125}I-lactoferrin binding studies with intact bacteria.	The data from this study establishes that lactoferrin binds to porins, a class of well-conserved molecules common in *E. coli* and many other gram-negative bacteria. However, in certain strains of *E. coli*, these pore forming proteins are shielded from lactoferrin interaction.	Erdei et al. 1994
The goal of this study was to confirm porin involvement in lactoferrin binding to Salmonella by simultaneously probing with a monoclonal antibody specific for a conserved porin domain (Pol) in different bacterial species of enterobacteriaceae and to examine the effect of lactoferrin interaction on the susceptibility of selected *Salmonella* species to antibiotics.	Interaction of both human and bovine lactoferrin with the cell envelope and outer membrane of *Salmonella typhiumurium* type strain ATCC13311 was confirmed to occur with a porin. Such lactoferrin binding porin proteins (37-39 kD range) were readily detected in nine other common Salmonella species. The antibiotic susceptibility of Salmonella in the presence of lactoferrin indicated that lactoferrin potentiates the effect of certain antibiotics.	Results further confirmed earlier reports of porin involvement in specific interaction of lactoferrin with bacterial members of the enterobacteriaceae family. Lactoferrin enhanced the susceptibility of bacteria for both types of antibiotics that cross the outer membrane either by a porin pathway or via a hydrophobic route. The affected antibiotics increased the antibacterial spectrum of bovine lactoferrin in an additive manner.	Naidu and Arnold 1994

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TABLE 18 Biological Functions Of Lactoferrin Antimicrobial Action			
Study Design	**Results**	**Comments**	**Reference**
The interaction of lactoferrin with *Actinobacilus actinomycetecomitans* was examined in a ^{125}I-labeled protein binding assay.	Binding of human and bovine lactoferrins reached maximum within 1 hour. Lactoferrin binding to the bacterium was pH dependent and reversible. Scatchard analysis indicated the existence of both high and low affinity binding sites. Bacteria in the exponential phase of growth showed higher binding than cells in the stationary phase. SDS-PAGE and Western blot analysis of the cell envelope as well as the outer membrane revealed lactoferrin reactive protein bands at 29 kDa and 16.5 kDa.	Data suggests a specific interaction of lactoferrin with outer membrane proteins of *Actinobacilus actinomycetecomitans.*	Alugupalli et al. 1995
The interaction of lactoferrin with *Aeromonas hydrophila* was tested in a ^{125}I-labeled protein binding assay.	The mean percent binding values for human and bovine lactoferrin were 13.4 ± 2.0 and 17.5 ± 2.7 (mean + SEM), respectively. The human and bovine binding reached a complete saturation within 2 hours. Unlabeled human and bovine lactoferrin displaced ^{125}I-human lactoferrin binding in a dose-dependent manner and more effectively by the heterologous (1 μg for 50% inhibition) than the homologous (10 μg for 50% inhibition) ligand. Apo- and holo-forms of human and bovine lactoferrin inhibited more than 80%, while mucin caused approximately 50% inhibition of the human lactoferrin binding. Various other proteins (including transferring) or carbohydrates did not block the binding. Two binding proteins were identified in the boiled cell envelope preparation while the unboiled cell envelope demonstrated a short-ladder pattern at the top of the separating gel and a second band at approximately 60 kDa position These corresponded to porins.	Data establish a specific interaction of lactoferrin with *Aeromonas hydrophila* and the lactoferrin binding proteins seem to be porins.	Kishore et al. 1991

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TABLE 18 Biological Functions Of Lactoferrin Antimicrobial Action			
Study Design	**Results**	**Comments**	**Reference**
The protective effect of lactoferricin, a potent antimicrobial peptide produced by gastric pepsin cleavage of lactoferrin, against *Toxoplasma gondii* infection was examined in experimental murine toxoplasmosis. Mice were administered 5.0 mg lactoferricin orally and challenged with cysts of *T. gondii* at a dose level of LD_{90}.	All mice orally administered lactoferricin survived until the end of the experiment (35 days post challenge). Intraperitoneal administration of 0.1 mg of lactoferricin also prevented death in 100% of treated mice challenged with *T. gondii* cysts. In contrast, 80% of untreated mice died of acute toxoplasmosis within 14 days post challenge. In the mice treated perorally with lactoferricin, the number of cysts in the brain was significantly lower than that in untreated mice. Levels of interferon-r in the serum of infected mice treated perorally with lactoferricin showed a tendency to be lower than those in the infected mice without treatment.	Results demonstrate that oral administration of lactoferricin induces resistance to *T. gondii* infection in mice.	Isamida et al. 1998

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2. Effects on Gastrointestinal Flora

The composition of the bacteria that comprise the gastrointestinal microflora have important ramifications for human health (Mitsuoka 1982; Drasar and Roberts 1989). Bifidobacteria are considered to be one of the most important genera of beneficial bacteria (Modler 1994; Gibson and Roberfroid 1995). The genus Bifidobacterium consists of at least 25 distinct species, 10 of whose main area of colonization is the human large intestine. In infants, bifidobacteria are one of the first bacterial groups to establish themselves in the intestinal tract and, within one week, become the predominant group (Hoover 1993; Mitsuoka 1982; Mitsuoka 1984; Poupard et al. 1973).

There are many purported beneficial effects of bifidobacteria on human health. Bifidobacteria may provide a defense against pathogenic bacteria. The predominance of the bifidobacteria in breast-fed infants is believed by many to afford some of the protection against enteral as well as systemic disorders caused by bacterial pathogens. It is thought that the predominance of bifidobacteria produces the lower morbidity and mortality seen among breast-fed infants (Yoshioka et al. 1983; Roberts 1986; Ogawa et al. 1992). Bifidobacteria have also been used therapeutically to restore the normal intestinal flora during antibiotic therapy (Gibson and Roberfroid 1995; Gibson et al. 1995; Wang and Gibson 1993; Ballongue 1998; Miller-Catchpole 1989).

Formula-fed babies normally have 1-log count less bifidobacteria present in fecal samples than human milk-fed babies. Formula-fed infants also generally have higher levels of enterobacteriaceae, streptococci, and anaerobes other than bifidobacteria (Hoover 1993; Yoshioka et al. 1983; Roberts 1986; Beerens et al. 1980). In addition, human milk-fed infants may be afforded more protection than formula-fed infants against many gastrointestinal infections. Investigations indicate that the protection is mainly due to antimicrobial, anti-inflammatory, and immunomodulating agents present in human milk that are not available from infant formulas. In addition to the control over the bacterial enteric flora that is exerted by direct-acting antimicrobial agents in human milk, human milk provides growth factors that encourage the proliferation of a protective enteric flora. One of the factors in human milk that is thought to promote the levels of bifidobacteria as well as inhibit the growth of selected pathogens in the colon is lactoferrin (Goldman et al. 1998).

In vitro studies (summarized in Table 19) demonstrate the ability of lactoferrin to promote the growth of bifidobacteria; this ability is independent of the iron-saturation level of lactoferrin. Bovine lactoferrin showed greater growth promotion of *B. infantis*

and *B. breve* while human lactoferrin was more active for *B. bifidum* (Petschow et al. 1999; Petschow and Talbott 1991).

Other *in vitro* investigations demonstrated bacteriostatic effects of lactoferrin against the pathogenic microorganisms *Listeria monocytogenes, Escherichia coli, Vibrio* cholerae, *Salmonella typhimurium,* and *Clostridium welchii* (Antonini et al. 1997; Bullen et al. 1972; Naidu et al. 1991; Ellison and Giehl 1991).

Several investigators have used human infants or animal (mouse) models to examine the effect of lactoferrin on microflora (summarized in Table 20). Most mouse studies analyzed the effect of bovine lactoferrin consumption at concentrations ranging from 0.5% to 5% of diet, administered in milk or milk-based infant formula diets. Because milk or formula consumption was not reported in the studies, the dose of lactoferrin on a mg/kg body weight basis can not be calculated. As a comparison, however, the concentration of lactoferrin administered can be compared to the lactoferrin concentration of human milk. Human milk contains approximately 2 mg/ml. The concentrations of lactoferrin administered in all but one of the mouse studies ranged from 0.005 mg/ml up to 0.05 mg/ml or 400 to 40 fold less than in human milk. One study in mice used a test formula which contained 2 mg/ml of bovine lactoferrin, the same concentration as in human milk.

When mice were fed milk unsupplemented with additional lactoferrin, the number of enterobacteria and bifidobacteria in the feces increased greatly (Teraguchi et al. 1995a; Teraguchi et al. 1994; Ogata et al. 1998). Addition of 1% to 2% lactoferrin to the milk significantly suppressed the proliferation of enterobacteria without affecting bifidobacteria relative to the levels seen on the milk diet (Teraguchi et al. 1995a; Ogata et al. 1998).

Bovine lactoferrin has been shown to promote the growth of certain bacteria such as *Bifidobacterium*, *Bacteroides*, and *Enterococcus* and total aerobes in the small intestine of germ-free mice colonized with predominant intestinal flora components isolated from the feces of breast-fed human infants (Hentges et al. 1992). The same pattern was observed with cecal homogenates except that the differences in bacterial counts were not statistically significant. Whether the finding of increased bifidobacteria is related to a growth promotion activity of lactoferrin or its ability to inhibit growth of other bacteria is not known.

The bacteriostatic effect of lactoferrin was clearly demonstrated *in vivo* against several strains of *Clostridium* (*C. ramosum, C. paraputrificum,* and *C. perfringens)* and *Enterobacteriaceae* (Teraguchi et al. 1994, 1995a,b). In general, the pepsin hydrolysate of bovine lactoferrin showed the same bacteriostatic effects on fecal bacteria as lactoferrin (Teraguchi et al. 1995a). A dose-response effect of bovine lactoferrin on the

inhibition of proliferation of *Enterobacteriaceae* was demonstrated at administered doses from 0.5% up to 2% bovine lactoferrin in SPF mice by Teraguchi et al. (1995a). In this study, SPF mice were fed bovine milk which produced a great increase in the number of fecal *Enterobacteriaceae* within two days. Subsequent lactoferrin administration resulted in a significant decrease in the fecal bacterial number with increasing lactoferrin concentrations up to 2%. Levels of *Enterobacteriaceae* remained constant for the groups fed milk containing more than 2% lactoferrin.

The effect of lactoferrin on the fecal microflora of infants was studied by Roberts et al. (1992). The supplementation of the diet with lactoferrin, however, was done at levels that are relatively low compared with human milk (lactoferrin given at 10 - 100 mg/100 ml compared to human milk levels of 200 up to 600 mg/100 ml). Some effect on enhancing bifidobacteria was seen in the high dose group but only at age 3 months. Balmer et al. (1989) also looked at the effect of bovine lactoferrin on fecal microflora of infants. Infants were given formula containing 2.8g/L lactoferrin (280mg/100ml). There was little effect upon the fecal microflora, however, and the pattern of the fecal flora was not the same as that of a breast-fed baby.

TABLE 19
Studies Investigating the *In Vitro* Effect of Lactoferrin on Intestinal Microflora

Objective	Study Design	Results	Reference
To investigate the role of bovine lactoferrin in protecting the intestinal epithelium from bacterial infections using an *in vitro* model.	Antibacterial activity of lactoferrin, adhesion and invasion of bacteria, and particle agglutination were investigated *in vitro* using human colonic cells, a strain of Listeria monocytogenes and various concentrations and types of bovine lactoferrin (iron saturated and unsaturated, and manganese-saturated.)	When infection occurred in the presence of 1 mg/ml of bovine lactoferrin, in the form of apolactoferrin, or iron- or manganese-saturated forms, the adhesion of bacteria to eukaryotic cells was unaffected, but the number of internalized bacteria was reduced by 42- to 125-fold. The possibility of a toxic effect of bovine lactoferrin was excluded since bovine lactoferrin under the same experimental conditions and concentrations did not exert any antibacterial activity.	Antonini et al 1997
To investigate whether the bifidobacteria growth promotion activity of lactoferrin is correlated with either the binding the lactoferrin to bifidobacterial cells or the iron saturation of lactoferrin.	Bifidobacteria were grown on a medium containing bovine lactoferrin and iron for 48 hrs. Acid production was measured as an indicator of bacterial growth and metabolism. Binding of lactoferrin to bacterial cells was determined using botinylated bovine lactoferrin and horseradish peroxidase-labeled streptavidin.	Bovine lactoferrin increased the growth of *B. infantis* and *B. breve in vitro* in a dose-dependent fashion, while much less growth promotion activity was observed for *B. bifidum*. Changes in the iron content of lactoferrin did not alter the bifidobacteria growth promotion activity of either bovine lactoferrin or human lactoferrin preparations. Competitive binding studies with biotinylated milk bovine lactoferrin showed that binding of bovine lactoferrin was inhibited by unlabelled bovine lactoferrin and human lactoferrin, but not by b-lactoglobulin, alpha lactalbumin, or transferrin. Binding of bovine lactoferrin to *B. bifidum* and *B. breve* was c. 40-fold higher than binding to *E. coli*. Collectively, the results demonstrate that the growth promotion activity of lactoferrin to Bifidobacterium spp. *in vitro* is independent of the iron saturation level of lactoferrin and suggest that binding of lactoferrin to bifidobacteria cells is not sufficient for stimulation of bifidobacterial growth.	Petschow et al 1999

TABLE 19 Studies Investigating the *In Vitro* Effect of Lactoferrin on Intestinal Microflora			
Objective	**Study Design**	**Results**	**Reference**
To investigate whether lactoferrin's ability to alter the gram-negative outer membrane of bacteria could enhance the antibacterial activity of lysozyme.	Human lactoferrin and lysozyme were used along with the bacterial strains *Vibrio cholerae* 3083, *E. coli* CL99-2, and *S. typhimurium* SK696 in time-kill studies, transmission electron microscopy (TEM), equilibrium dialysis studies with calcium, and LPS binding studies with several additional iron-binding proteins.	While protein and lysozyme alone are each bacteriostatic, together they can be bactericidal for strains of *V. cholerae*, *S. typhimurium*, and *E. coli*. The bactericidal effect is dose dependent, blocked by iron saturation of lactoferrin, and inhibited by high calcium levels, although lactoferrin does not chelate calcium. The effect of lactoferrin and lysozyme can be inhibited with increasing media osmolarity. TEM shows that *E. coli* cells exposed to lactoferrin and lysozyme at 40mOsm become enlarged and hypodense, suggesting killing through osmotic damage. Dialysis chamber studies indicate that bacterial killing requires direct contact with lactoferrin. Work with purified LPS suggests that this relates to direct LPS-binding by the protein.	Ellison & Giehl 1991
To compare the growth promotional activity of protein and non-protein components of human milk and cow milk on infant strains of Bifidobacterium species.	Various strains of Bifidobacterium were grown on several medias under anaerobic conditions for 48 hrs with or without test sample materials (human milk (HM), cow's milk CM, bovine lactoferrin, BSA, α-LA, β-LG). Acid production was measured as an indicator of bacterial growth.	HM samples varied considerably in growth-promotion activity for *B. bifidum* var *pennsylvanicus*, *B. infantis*, and *B. breve*. CM samples showed similar results with less variability. Activity in CM whey was found in both protein nitrogen and non-protein nigrogen fractions. Bovine lactoferrin and α-LA showed the greatest growth-promotion activity in *B. infantis* and *B. breve*, even at levels as low as 0.25 mg/ml, but did promote growth in all 5 strains of Bifidobacterium examined. 2-4 ml/mg α-LA or bovine lactoferrin were required to promote growth in *B. bifidum* var *pennsylvanicus* and B. *longum*.	Petschow & Talbott 1991

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TABLE 19 Studies Investigating the *In Vitro* Effect of Lactoferrin on Intestinal Microflora			
Objective	**Study Design**	**Results**	**Reference**
To investigate the bacteriostatic effect of human milk and bovine colostrum and purified human lactoferrin with specific bacterial antibody.	Milk or lactoferrin samples with varying levels of iron saturation were innoculated with bacteria (*E. coli, C. welchii, L. bifidus*) and viable counts of bacteria were determined.	Three samples of human milk with unsaturated iron-binding capacities of between 56 and 89% had a powerful bacteriostatic effect on *E. Coli* 111/B4. The bacteriostatic properties of milk were diminished if the iron-binding proteins were saturated with iron. Purified human lactoferrin, in combination with specific *E. coli* antibody, strongly inhibited the growth of *E. coli*, and this effect was also diminished by saturating the lactoferrin with iron.	Bullen et al 1972
To investigate and characterize a specific binding of lactoferrin to *E. coli* and to seek a correlation between lactoferrin binding and different clinical groups or serotypes of *E. coli*.	Bovine and human lactoferrin were labeled and incubated with over 200 different strains of *E. coli*. Radioactivity of bound lactoferrin was measured and expressed as a percentage of the total labeled lactoferrin added to the bacteria.	The human lactoferrin and bovine lactoferrin binding to *E. coli* was in the range 3.7 to 73.4% and 4.8 to 61.6%, respectively. Enterotoxigenic strains demonstrated a significantly higher human lactoferrin binding than enteropathogenic, enteroinvasive, enterohaemorrhagic strains or normal intestinal *E. coli* isolates. No significant differences in the degree of human lactoferrin or bovine lactoferrin binding were found between aerobactin-producing and non-producing strains. The results demonstrate a specific binding of *E. coli* to lactoferrin, an acute-phase, iron-binding, antimicrobial host component.	Naidu et al 1991

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TABLE 20
Studies Investigating the *In Vivo* Effect of Lactoferrin on Intestinal Microflora

Species	Objective	Study Design	Results	Reference
Mice	To investigate the effect of a milk diet and bovine lactoferrin (bLF) or a pepsin-generated hydrolysate (bLFH) of bovine lactoferrin on the interaction between bacterial translocation and intestinal bacteria.	Six groups of five mice (5 wks of age) were fed one of the following test diets for 7 days: milk containing bLF at a concentration of 0, 0.5, 1.0, or 2%, or milk containing 2% bLFH. The control group received pelleted rat chow. Five groups of 10 mice each were either pellets, milk, or milk supplemented with 2% bLF, 2% bLFH, or 2% casein sodium for 7 days. The mice were then killed and the organs removed. Bacterial CFUs were assayed in the mesenteric lymph nodes, liver and spleen.	When mice were fed milk with no lactoferrin supplementation, the number of enterobacteria in the feces increased greatly, along with an increase in streptococci, lactobacilli, bifidobacteria, and clostridia compared to the pellet control. Addition of 1% or 2% bLF or 2% bLFH to the milk significantly suppressed the proliferation of fecal enterobacteria compared with unsupplemented milk. Translocating bacteria were isolated from the MLN of all the groups at various rates of incidence but never from the spleen or liver. Feeding of bovine milk to mice induced a high incidence of bacterial translocation from the intestines to the mesenteric lymph nodes, and the bacteria involved were mainly members of the family *Enterobacteriaceae*. Supplementation of the milk diet with bovine lactoferrin or a pepsin-generated hydrolysate of bovine lactoferrin resulted in significant suppression of bacterial translocation. No substantial effect of bLF on the small intestine was observed in relation to length, tissue weight, mucosal weight, mucosal protein, or DNA content. The epithelial barrier appeared to be intact and to have normal architecture in all of the groups.	Teraguchi et al., 1995a.
Mice	To determine the effect of diet on intestinal ecology in germ-free (GF) mice that were inoculated orogastrically	GF mice were colonized *with Bifidobacterium bifidum*, *Bacteroides*, *Clostridium bifermentans*, *Enterococcus faecalis*, *E. coli*, and *S. epidermidis*. Groups of flora-associated mice (5 mice/group) were fed	Addition of bovine lactoferrin (bLF) to the formula resulted in significantly greater counts of *Bifidobacterium*, *Bacteroides*, *Enterococcus* and total aerobes in the small intestine when compared with mice fed unsupplemented	Hentges et al 1992

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TABLE 20 Studies Investigating the *In Vivo* Effect of Lactoferrin on Intestinal Microflora				
Species	**Objective**	**Study Design**	**Results**	**Reference**
	with predominant intestinal flora components isolated from the feces of breast-fed human infants.	either human milk, bovine milk, whey-dominant formula, or modified formula (one of which contained 2 mg/ml bovine lactoferrin) for 2 weeks. Mice were then examined for changes in small intestinal and cecal flora composition, cecal pH and resistance to intestinal colonization with *Salmonella typhimurium*.	formula. *Bifidobacterium* was present in large numbers in both the ceca and small intestines of mice fed the lactoferrin formula. Consumption of human milk resulted in a lower pH of the cecal contents and a greater resistance to colonization by *Salmonella typhimurium* after orogastric challenge than the consumption of the other diets. Regarding these last two effects, the addition of bLF to formula did not result in a significant difference when compared to unsupplemented formula.	
Mice	The *in vivo* mechanism of bacteriostasis of bLF was investigated with the aim of further elucidating the biological role of lactoferrin in mammalian milk. The influence of administered carbohydrate and bLF on the proliferation of intestinal bacteria in the gut of SPF mice fed a milk diet was examined. Also examined was the stimulation effect of bLF on the absorption of carbohydrates *in vivo* and *in vitro*.	Five-week old mice were used in all experiments. To study the influence of carbohydrates and bLF on intestinal bacteria, groups of 5 SPF mice were fed one of eight diets: bovine milk (4% lactose, milk treated with beta-D-galactosidase (2% glucose and 2% galactose), a mixture of the previous two milks, milk supplemented with lactose (7% lactose), or one of the previous milks supplemented with 2% bovine lactoferrin (bLF). After 7 days the bacterial numbers in the feces were assayed for *Enterobacteriaceae* and bifidobacteria. To study the carbohydrate absorption in germ-free (GF) mice, two groups of 12 GF mice were fed either sterile milk or milk supplemented with 2% bLF for 7 days. Contents of the cecum were assayed for lactose, glucose, and galactose. Mucosal disaccharidase activity was measured from the	The proliferation of intestinal *Enterobacteriaceae* was consistent among the SPF mice fed milk diets and appeared to be independent of the carbohydrate concentration in the diet. The proliferation of *bifidobacteria* was dependent on the presence of lactose in the diets. The addition of 2% bLF to the diets significantly suppressed the proliferation of enterbacteria except in the case of the diets containing 7% lactose. The bLF also suppressed bifidobacteria in the group of mice fed 2% lactose, but not in the groups fed 4 or 7% lactose. In GF mice fed sterile milk, addition of 2% bLF to milk resulted in a significant decrease in concentrations of lactose, glucose, and galactose in the cecal contents. In an *in vitro* assay system, both bLF and its pepsin hydrolysate stimulated glucose absorption.	Ogata et al 1998

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TABLE 20 Studies Investigating the *In Vivo* Effect of Lactoferrin on Intestinal Microflora				
Species	**Objective**	**Study Design**	**Results**	**Reference**
		small intestine. To study carbohydrate absorption *in vitro*, sacs of everted small intestine from pellet-fed SPF mice were assayed. The glucose absorption rate was estimated from the increase in glucose concentration in the PBS or its decrease in the reaction mixture.	The authors propose that the *in vivo* mechanism of action of ingested bLF involves the stimulation of glucose absorption resulting in a bacteriostatic effect against *Enterobaceriaceae* in the intestine of milk-fed mice.	
Mice	1) To study the influence of a bovine milk diet with and without added bovine lactoferrin (bLF) on fecal *Enterobacteriaceae.* 2) To examine the effects of bLFs with varying iron saturation on the initial proliferation of fecal *Enterobacteriaceae* and on gastrointestinal *Enterobacteriaceae.* 3) To examine the effects of bLF concentration on fecal *Enterobacteriaceae.* 4) To determine the effects of various proteins including bLF on fecal *Enterobacteriaceae.*	1) Four groups of 10 mice each were fed one of the following diets: pellets plus water for 30 days; pellets plus 5% bovine lactoferrin water solution during days 8 to 22 but pelleted diet on other days; bovine milk for 30 days; milk with 5% bLF on days 8 to 22 with unsupplemented milk on other days. Feces were collected at various intervals and assayed for *Enterobacteriaceae.* 2) Five groups of 10 mice each were fed one of the following diets for 14 days: pelleted diet and water; bovine milk; milk plus 5% native bLF; milk plus 5% apo-bLF (iron saturation 2.3%); milk plus 5% holo-bLF (iron saturation 97.6%). Two mice per group were killed and gastrointestinal trace removed. 3) Five groups of 10 mice each were fed one of the following diets: bovine milk containing native bLF at concentrations of 0, 0.5, 1.0, 2.0, or 5.0%. Feces collected after 11 and 18 days and assayed for *Enterobacteriaceae.* 4) Eight groups of five mice each were fed a pelleted diet, bovine milk, or milk containing	The number of fecal *Enterobacteriaceae* increased greatly in SPF mice fed bovine milk compared to SPF mice fed a pelleted diet. *E. coli* and *Proteus mirabilis* were the dominant Enterobacter species isolated from mice feces. When mice were fed milk with bovine lactoferrin the fecal enterobacteria number decreased to the level found in the feces of mice fed the pelleted diet. Administered bLF caused suppression of the initial proliferation of fecal *Enterobacteriaceae* in the milk-fed mice. The bacteriostatic effect of bLF was independent of the iron-chelating ability of bLF. The bacteriostatic effect of administered bLF was dependent on the concentration of bLF and the duration of feeding. The number of fecal enterobacteria decreased with bLF concentrations from 0.5% to 2% and remained constant for the groups fed milk containing more than 2% bLF. The number of enterobacteria was statistically significantly reduced with lactoferrin feeding compared to unsupplemented bovine milk feeding.	Teraguchi et al. 1994

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TABLE 20 Studies Investigating the *In Vivo* Effect of Lactoferrin on Intestinal Microflora				
Species	**Objective**	**Study Design**	**Results**	**Reference**
		2% of one of the following compounds: native bLF, bovine serum albumin, bovine casein, bovine whey protein isolate which contained less than 1% bLF, pepsin hydrolysate of bLF. After 7 days, feces were collected and assayed for *Enterobacteriaceae*.	A bacteriostatic effect was also observed in each part of the gastrointestinal tract of mice fed bLF-containing milk. The pepsin hydrolysate of bLF showed the same effect on fecal bacteria as native bLF. The effect of milk containing ovalbumin, whey protein isolate, or casein was the same as that of mice fed only milk.	
Mice	To investigate whether bLF or bLFH in milk fed to mice exerts a suppressive effect on *in vivo* proliferation of orally administered *Clostridium* species. Different species of *Clostridium* were tested in a similar manner to determine whether ingested bLF exerts a common effect against various intestinal clostridia.	4-wk old female BALB/c SPF mice were used in all experiments. 1) *In vivo* proliferation of *C. ramosum*. Four groups of 5 mice each were dosed with either 7.0 log10 CFU or 5.0 log 10 CFU using an oral catheter. The groups were then fed bovine milk or pellets ad libitum. Feces were assayed for *C. ramosum*. 2) Influence of bLF on fecal microflora. Each of nine mice was administered 7.0 log10 CFU *C. ramosum* and then fed bovine milk for 7 days followed by milk plus 2% bLF for 7 days. Feces were assayed for *C. ramosum*. 3) Effects of bLF and bLFH on proliferation of *C. ramosum*. Five groups of five mice each were administered 7.0 log10 CFU C. For 7 days before and 7 days after inoculation, mice were fed one of the following diets: milk only, milk followed after inoculation by milk plus 2% bLF, milk containing 2% bLF only, milk containing 2% bLFH only, or pellets only. Feces were assayed for *C. ramosum*. 4) Effect of bLF concentration. Eight groups	1) When milk-fed mice were orally inoculated with *Clostridium ramosum* C1, this strain proliferated in the gut and became the dominant component of the fecal microflora. 2) Ingestion of milk containing bovine lactoferrin suppressed the proliferation of *Clostridium in vivo* and decreased the numbers of total bacteria and the specific bacteria, *Enterobacteriaceae*, *Streptococci*, and *Lactobacilli*, in the feces compared to milk without added bovine lactoferrin. Bifidobacteria numbers were increased substantially with both milk and milk plus lactoferrin feedings compared to pellet control. There was no difference, however, between the milk or the milk plus lactoferrin feedings on bifidobacteria numbers. 3) *C. ramosum* was detected in the feces of mice fed milk, but not in the feces of the group fed pellets. The numbers of *C. ramosum* in feces from the three groups fed 2% bLF or 2% bLFH were significantly less than those of the group fed milk only. 4) The bacteriostatic effect of bLF was	Teraguchi et al. 1995b

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TABLE 20 Studies Investigating the *In Vivo* Effect of Lactoferrin on Intestinal Microflora				
Species	**Objective**	**Study Design**	**Results**	**Reference**
		of five mice each were fed bovine milk containing bLF at a concentration of 0.5, 1.0, 2.0% for 14 days. After 7 days groups were administered either 7.1 or 5.2 log10 CFU *C. ramosum C1*. 5) Specificity of the bacteriostatic effect of bLF. Seven groups of 5 mice each were fed for 14 days bovine milk or milk containing one of the following proteins at a concentration of 2%: bLF, bLFH, bovine serum albumin, ovalbumin, bovine casein, or bovine whey protein isolate. After 7 days each group was administered 5.0 log10CFU of *C. ramosum C1*. 6) Effects of bLF on various *Clostridium* species. Five groups of five or ten mice each were fed bovine milk. Five additional groups were fed milk containing 2% bLF for 14 days. After 7 days, one group fed milk and one group fed milk containing 2% bLF were administered a suspension containing multiple *Clostridium* strains.	dependent on the concentration of bLF, the duration of feeding, and the administered dose of *C. ramosum* C1. The numbers of fecal *C. ramosum* significantly decreased with bLF concentrations from 0.5% to 2%. At 2% bLF, the fecal numbers in the group administered 5.2 log10 CFU of *C. ramosum* C1 decreased significantly compared with the group administered 7.1 log10 CFU. 5) Compared with bovine serum albumin, ovalbumin, bovine whey protein isolate, or bovine casein, only bLF showed a bacteriostatic effect against *C. ramosum* in the gut. A hydrolysate prepared by digestion of bLF with porcine pepsin showed the same inhibitory effect on proliferation of *C. ramosum in vivo* as occurred with undigested bLF. 6) Following inoculation, a bacteriostatic effect from 2% bLF was observed with *C. ramosum* JCM 1298, *C. paraputrificum* VP1 6372, or *C. perfringens* ATCC 13124.	

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TABLE 20 Studies Investigating the *In Vivo* Effect of Lactoferrin on Intestinal Microflora				
Species	**Objective**	**Study Design**	**Results**	**Reference**
Human infants	The objective of the study was to investigate the effects of lactoferrin on the fecal flora.	Formula was given to babies for fourteen days. Formulas used were (a) no added iron and no added lactoferrin (n=20); (b) no iron but added lactoferrin (n=18); (c) added iron and lactoferrin (n=20). Lactoferrin was added at 2.8 g/L formula. Anthropometric measurements were made on day 1. Feces were collected on days 4, 11 and 14. Specimens collected on days 4 and 14 were used to measure the bacterial flora and the 24 hour specimen collected on day 11 was used to estimate fecal lactoferrin concentration.	The addition of lactoferrin had little effect upon the fecal microflora and did not move the pattern of the fecal flora in the direction of the breast fed baby.	Balmer et al. 1989

TABLE 20 Studies Investigating the *In Vivo* Effect of Lactoferrin on Intestinal Microflora				
Species	**Objective**	**Study Design**	**Results**	**Reference**
Human infants	The objective of the study was to investigate the development of the fecal flora in infants given lactoferrin supplemented formula.	Four infant feeding groups were studied. Fifty one infants finished the study and a further four infants provided two samples during the study period. The subjects were fed on the following diets: group A, breast feeding, 12 infants; group B, formula feeding, 14 infants; group C, experimental formula supplemented with 10 mg/100 ml lactoferrin, 15 infants; and group D, experimental formula supplemented with 100 mg/100 ml lactoferrin, 14 infants. Study duration was 3 months.	Breast fed infants developed a flora rich in Bifidobacteria. Facultative anaerobes were ubiquitous, but in relatively small numbers within the diet group. Other obligate anaerobes, such as *Clostridium* sp. and *Bacteroides* sp. were rarely isolated. Standard formula produced a flora rich in bifidobacteria, but the growth of facultative organisms was not suppressed by this diet. *Clostridium* sp. and *Bacteroides* sp. were more common in this feeding group. The addition of 10 mg lactoferrin/100 ml formula produced a flora similar to that of the standard formula fed babies. Lactoferrin at 100 mg/100 ml was able to establish a "bifid flora" in half of the babies given this formula, but only at age three months. *Clostridium* sp. and *Bacteroides* sp. were common isolates from babies receiving both the lactoferrin diets. No adverse health effects of lactoferrin consumption were reported. The formula was supplemented with lactoferrin at levels that are relatively low compared with human milk (200-600 mg/100 ml milk). This may have contributed to the lack of effect of lactoferrin on the fecal flora. In addition, the fact that the lactoferrin was 20% saturated with iron may have negated or weakened its effect.	Roberts et al. 1992

V. ANALYTICAL METHODOLOGY

Measurements of lactoferrin in food can be determined by several methods (Appendix E). Shinmoto et al. (1997) describes a competitive ELISA, which uses affinity-purified bispecific antibodies that are sensitive enough to measure bovine lactoferrin concentrations in milk, saliva and plasma. Molecular cloning and sequence analysis has been used to identify and sequence bovine lactoferrin isolated from bovine submaxillary glands (Pierce et al. 1991). Gel electrophoresis like SDS-PAGE can also be used to separate lactoferrin based on molecular particle size (Pharmacia).

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VI. SAFETY ASSESSMENT

A. Fate of Lactoferrin in the GI Tract

After gastric emptying, digestion products are subjected to the action of pancreatic enzymes and subsequent membrane peptidase hydrolysis and absorption along the small intestine. Proteins, according to both their structure and the nature of the chyme, are degraded differently by gastric and pancreatic proteases into peptides and amino acids and taken up by the intestinal mucosa, where brush border membrane peptidases and transport systems are responsible for the ultimate transfer of amino acids to the blood. Study of the overall digestion and absorption process of milk proteins show that gastric emptying appears to be the major mechanism controlling the absorption kinetics of nitrogen from milk (Tomé and Debabbi 1998).

Some dietary proteins, however, may elude the luminal hydrolytic process and reach the intestinal mucosa in significant amounts, where they are subsequently absorbed through different mechanisms. Transcytosis of intact protein was detected for both β-lactoglobulin, α-lactalbumin, prolactin and lactoferrin but not for casein (Tomé and Debabbi 1998). The fact that lactoferrin is only slowly degraded, as demonstrated in the piglet (Schmitz et al. 1989); baby monkey (Lindberg et al. 1997), and human newborn (Lindberg et al. 1998; Davidson and Lönnerdal 1987, 1985; Spik et al. 1982), is of particular interest from a physiological perspective. Very limited proteolysis in the stomach might actually release fragments of lactoferrin that have more potent bactericidal activity than the native molecule (Hamosh 1998).

A review of the studies examining lactoferrin fate in the gastrointestinal tract is presented in Table 21. Studies in human infants demonstrate that considerable quantities of intact lactoferrin are excreted by the breast-fed infant. Higher amounts are excreted during the early weeks of life and the quantity excreted is progressively smaller during successive weekly measurements (Davidson and Lönnerdal 1987, 1985). During the first weeks of life, approximately 2 to 6% of the lactoferrin consumed by the infant is excreted in the feces per day, however, by 4 months, less than 2% of the lactoferrin intake is excreted. Increased proteolysis of the lactoferrin takes place as the infant ages. The lactoferrin excreted in the feces was demonstrated to be intact (Davidson and Lönnerdal 1987). The large amounts of lactoferrin excreted in the feces intact in infants, along with the finding in this study that no other major whey proteins are detected in the feces, reinforces the fact that lactoferrin is protected against degradation. This is consistent with its purported physiologic role as an antimicrobial agent in the digestive system of the infant. Two studies of lactoferrin excretion in infants fed formula containing supplemental lactoferrin (2.8 g/L and 285 mg/L) (Balmer et al. 1989; Fairweather-Tait et al. 1987) indicated that as expected, babies fed formula containing bovine lactoferrin excreted significantly more

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lactoferrin in their stools than babies fed the basic formula. The levels excreted, however, were significantly lower than levels excreted from breast-fed babies. Most of the lactoferrin from the formula appeared to have been broken down.

Intact lactoferrin has also been found in the urine of human milk-fed preterm infants. Because serum levels of lactoferrin in both preterm and term infants do not vary with diet (i.e., human milk vs. bovine milk based formula), Hutchens et al. (1991) conducted a study to determine the origin of the lactoferrin in urine. In this study, it was determined that nearly all of the intact lactoferrin in the urine is of maternal origin. The physiologic significance of absorbed, undegraded lactoferrin has not yet been determined. In a similar study by Goldman et al. (1990), two groups of very low birth weight infants were investigated; one group received cow's milk formula and the second received fortified human milk. Lactoferrin was not found in excreta from infants fed cow's milk formula; however, intact and fragmented forms of lactoferrin were found in stools and concentrated urine of each infant who received human milk. The close resemblance between the lactoferrin fragments in the stools and urine suggest that the urinary lactoferrin fragments originated in the GI tract.

In contrast to the findings of Balmer et al. (1989) and Fairweather-Tait et al. (1987), a comparison of the fate of bovine lactoferrin and human lactoferrin fed to healthy babies age 3 days to 2 months showed that bovine lactoferrin is less degraded than the human forms and total excretion per day of the intact protein was greater (Spik et al. 1982). The iron saturated form was more resistant to degradation. Quantitative determination of human copro-lactoferrin from birth through a period of up to 3 weeks showed that the daily elimination decreased from 25 to 5 mg. Amount of copro-lactoferrin corresponding to the endogenous secretion (biosynthesis by the intestinal tract) was calculated to be from 0.5 to 1 mg/day.

The mean plasma level of lactoferrin in adults is 122 ± 40 μg/L; in term infants up to the age of 21 days, mean lactoferrin level is 385 ± 113 μg/L. The difference in the means between adults and infants is statistically significant. There were no significant differences between the means of breast-fed and formula-fed infants. No significant differences in plasma levels were found between adults and infants at 15 weeks of age (Scott 1989).

The plasma lactoferrin concentration in adults is derived from neutrophils. Lactoferrin derived from breast milk, given to adults, is rapidly cleared from the circulation, being avidly sequestered by the liver and spleen. The sequestered lactoferrin is promptly catabolized. The iron, however, is not excreted in the urine, but rather retained and slowly transferred to the bone marrow, where it is subsequently incorporated into developing erythrocytes (Bennett and Kokocinski 1979).

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TABLE 21 Fate of Ingested Lactoferrin in the Body			
Study Design	**Results**	**Conclusions**	**Reference**
Fecal samples were collected from exclusively breast-fed term infants and milk samples were collected from mothers. The first collection was taken soon after return from the hospital, within the first week of age. Thereafter, samples were collected once a week for the first month of life, then once a month for as long as the infant was exclusively breast-fed. Soluble proteins in the feces were extracted and analyzed for total protein, nitrogen, lactoferrin, secretory IgA, serum albumin and lysozyme.	Considerable quantities of lactoferrin were excreted by the breast-fed infant, with higher amounts excreted during the early weeks of life and the quantity excreted getting progressively smaller at successive weekly measurements. Quantities excreted for the three infants ranged from: 153 - 24 mg/24 hours (infant #1 from weeks 1 - 16); 46 - 12 mg/24 hours (infant #2 from weeks 3 - 22) and 70 - 9 mg/24 hours (infant #3 from weeks 2 - 12). This corresponded to concentrations of lactoferrin in the milk which were highest during the first weeks of life and decreased in concentration during later weeks. Gel filtration chromatography demonstrated the excreted lactoferrin to be intact. Fragments of these proteins were not detectable by immunoelectrophoresis following gel filtration. (Serum albumin, a major whey protein which can be assumed to be completely utilized by the infant, was undetectable in all of the fecal extracts.) Using milk consumption by the infant along with concentration of the specific proteins in the milk, 24 hour intakes of lactoferrin were calculated. Intakes by infants #1 and #2 ranged from 2,461 - 1,456 mg/24 hours (weeks 1 - 16) and 3,537 - 1,829 mg/24 hours (weeks 1 - 12), respectively. The percent of lactoferrin ingested that was excreted in the feces ranged from 6.2% - 1.3% in infant #1 and 2.0 - 0.4% in infant #2 during these same time periods.	This study examined the normal protein excretion from full-term exclusively breast-fed infants. Of the protein excreted by the infants, lactoferrin and IgA make up a significant proportion. Fecal lactoferrin is detectable throughout the study. Lactoferrin is believed to provide additional protection in the infant gut against several microorganisms. In its iron-saturated form, it is quite resistant to proteolysis. During the first weeks of life, approximately 2 - 6% of the lactoferrin consumed by the infant is excreted per day, however by 4 months, less than 2% of the lactoferrin intake is excreted. Increased lactoferrin proteolysis takes place as the infant ages. The large amounts of lactoferrin and IgA excreted intact by the infant, along with the fact that no other major whey proteins is detected in the feces reinforces the fact that lactoferrin and secretory IgA are protected against degradation, which is consistent with their physiological roles in the infants' digestive system.	Davidson and Lönnerdal (1987)

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TABLE 21
Fate of Ingested Lactoferrin in the Body

Study Design	Results	Conclusions	Reference
The origin of intact (78-kD) lactoferrin found in the urine of human milk-fed preterm infants was investigated using human milk containing proteins enriched with [^{13}C]leucine and [$^{15}N_2$]lysine or [$^{2}H_4$]lysine. Labeled milk was collected from each mother, pooled, fortified with a lyophilized human milk fraction, and fed to her preterm infant by continuous orogastric infusion for a period of 48 hours. Urine was collected from each infant for 96 hours.	Mass spectral analyses indicated that the isotopic enrichment of the purified urinary lactoferrin was 87 to 100% of that in the labeled human milk lactoferrin. Similar results were obtained for the isolated DNA binding lactoferrin fragments. The ratios of isotopically labeled leucine to lysine in the purified milk lactoferrins and urinary lactoferrins were similar for each mother/infant pair. Isotopically labeled lysine, added to the milk as free amino acid, was not incorporated into the purified urinary lactoferrin. These results demonstrate that undegraded lactoferrin of maternal origin is absorbed by the gut and excreted intact in the urine of preterm infants; nearly all the urinary lactoferrin was of maternal origin.	Serum levels of lactoferrin in both preterm and term infants reportedly do not vary with diet (i.e., human milk vs. bovine milk based formula). As a result, the origin of biologically significant quantities of lactoferrin in the urine of preterm infants fed human milk has been the subject of debate. Because fecal excretion of lactoferrin accounts for only 1 - 6% of the dietary lactoferrin in preterm infants, it has been suggested that much of the remaining lactoferrin is digested. This study demonstrated that nearly all of the intact lactoferrin in the urine of premature infants fed human milk is of maternal origin. The physiologic significance to preterm infants of absorbed, undegraded lactoferrin remains to be established. It uptake may proceed by nonspecific macromolecular absorption or by way of association with specific lactoferrin receptors.	Hutchens et al. 1991
Forty healthy babies aged 3 days to 2 months were fed on 6 different kinds of regimes: Group I, 15 babies were completely breast fed; Group II, 5 babies were completely bottle fed on a commercial concentrated and homogenized cow's milk; Group III, 5 babies were bottle fed on this formula for 2 days and then	Quantitative determination of human copro-lactoferrin from birth through a period of up to 3 weeks showed that the daily elimination decreased from 35 to 5 mg. The amount of copro-lactoferrin corresponding to the endogenous secretion (biosynthesis by the intestinal tract) was calculated to be from 0.5 to 1 mg/day. When a cow's milk diet was supplemented by partially or completely iron-saturated human or bovine lactoferrin and fed to the babies, the amounts of copro-lactoferrin excreted depended upon the origin and iron saturation of the lactoferrin. Total excretion (mg/24 hours) of	Data from this study showed that unhydrolyzed lactoferrin may be found in fecal extracts of babies. The fact that bovine lactoferrin is less degraded than the human form suggests that it may also have a powerful bacteriostatic effect in the infant gut and cold be used to supplement artificial formula.	Spik et al. 1982

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TABLE 21 Fate of Ingested Lactoferrin in the Body			
Study Design	**Results**	**Conclusions**	**Reference**
bottle fed on cow's milk with added 200 mg of partially iron-saturated lactoferrin at each of the 5 feeds/day for 3 days and again bottle fed on cow's milk for 2 days; Groups IV, V and VI, 5 babies in each group, were treated the same as Group III, except that the cow's milk was enriched by human iron-saturated lactoferrin for group IV, by bovine partially iron-saturated lactoferrin for Group V and by bovine iron-saturated lactoferrin for group VI. Lactoferrin was characterized in the fecal extracts.	lactoferrin in the stools of infants receiving the cow's milk supplemented by: human lactoferrin, ranges from 8 to 20 for the native lactoferrin and 21 to 37 for the saturated lactoferrin; and bovine lactoferrin, ranges from 62 to 220 for the native lactoferrin and 120 to 364 for the saturated lactoferrin. The iron saturated forms appeared to be more resistant to proteolytic degradation. Fingerprints of native human and bovine lactoferrins hydrolyzed *in vitro* by infant's gastric or duodenal secretions showed that both proteins were not extensively digested.		
Blood was obtained from 23 infants between 24 and 36 weeks of age. Blood was obtained within 24 hours of birth. Plasma lactoferrin measurements were made.	Plasma lactoferrin concentrations ranged from below to above those found in term infants. Plasma lactoferrin ranged from approximately 50 - 900 μg/L. Analysis of increase in lactoferrin concentration (> 200 μg/L in less than 48 hours) with clinical judgment of infection, indicated that there was a substantial degree of agreement. Infection was correlated with increased lactoferrin concentration.	The process of phagocytosis by circulating neutrophils has been shown to involve the release of several proteins, one of which is lactoferrin. Lactoferrin concentrations in preterm babies may rise transiently, such increases often being associated with clinical signs of infection.	Scott 1989
Two groups of very low birth weight infants were investigated; one group received cow's milk formula and the second received fortified human milk prepared daily by adding pasteurized, lyophilized mature donor milk to fresh maternal milk. Stools and urine were collected continuously during the 96 hour balance	Lactoferrin was not found by Western blotting in excreta from infants fed cow's milk formula. In contrast, intact and fragmented forms of lactoferrin were detected in stools and concentrated urine of each infant who received human milk. Only intact lactoferrin was detected in the fortified human milk preparation, whereas many types of lactoferrin fragments were present in the stools and urine. The approximate molecular weight of the most prominent fragments were 44, 38, 34 and 32 kD. However, the stools also displayed lower molecular weight fragments that were not found in urines of those infants. These	Fragments of lactoferrin in stools of very low birth weight infants fed human milk appeared to be produced by *in vivo* proteolysis. The close resemblance between the lactoferrin fragments in the stools and urine suggest that the urinary lactoferrin fragments originated in the GI tract.	Goldman et al. 1990

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TABLE 21 Fate of Ingested Lactoferrin in the Body			
Study Design	**Results**	**Conclusions**	**Reference**
periods at 2.5 and 5 weeks of age.	fragments were similar in size to those produced *in vitro* by limited digestion of apo-lactoferrin with trypsin.		
Plasma lactoferrin was measured using a non-isotopic enzyme immunoassay in normal full term infants up to 15 weeks of age and in normal adults. Values of plasma lactoferrin were also compared in exclusively breast-fed infants with those receiving feeding formulas.	Venous blood specimens were obtained from 21 adults. Mean adult plasma lactoferrin value was 122 ± 40 µg/L (range of 42 - 202). Capillary specimens from 13 adults gave a mean plasma lactoferrin value of 107 ± 73 µg/L. For infants, 175 capillary blood specimens were obtained. Mean plasma lactoferrin from term infants up to the age of 21 days was 385± 113 µg/L, which was significantly higher than that found in adult plasma. Plasma lactoferrin values at 7, 11 and 15 weeks gave means of 267 ± 176 µg/L, 269 ± 163 µg/Land 176 ± 165 µg/L, respectively. Means at 7 and 11 weeks were significantly higher than in adults, however no significant difference was found at 15 weeks. There were no significant differences between the means of breast-fed and formula-fed groups.	The infant plasma lactoferrin concentrations remained significantly higher than the adult up until 15 weeks. However, the concentration of lactoferrin in cow's milk feeding formula is low (20 - 200 mg/L) compared to human milk (approximately 1 g/L in mature milk), however there was no effect of feeding type on plasma lactoferrin levels.	Scott 1989
Fecal samples from breast-fed infants and milk samples were collected for 24 or 48 hours.	Milk from donor #1 contained 1.3 mg/ml lactoferrin. Fecal samples from this mother's infant contained 14 mg lactoferrin/24 hours at 15 weeks. Milk from donor #2 contained 1.8 mg/ml lactoferrin. Fecal samples from this mother's infant contained 8.0 mg lactoferrin/24 hours at 15 weeks. Lactoferrin constituted a fairly large proportion of fecal protein (32% for infant #1 and 19% for infant #2).	Lactoferrin is found in high concentration in breast milk compared to cow's milk and infant formula. It is thought to act as a bacteriostatic agent through the GI tract of the infant. This proposed role necessitates the persistence of the protein through at least part of the GI tract of the infant.	Davidson and Lönnerdal 1985
Formula was given to babies for fourteen days. Formulas used were (a) no added iron and no added lactoferrin (n=20); (b) no iron but added lactoferrin (n=18); (c) added iron and lactoferrin (n=20). Lactoferrin was added at 2.8 g/L formula. Anthropometric measurements were made on day 1. Feces were collected on days 4, 11 and 14. Specimens collected on days 4 and 14 were used to measure the bacterial flora	Lactoferrin excretion in the feces was greater in the lactoferrin fed babies than in those fed the basic formula, however, observed excretion was far below that expected. Reports of lactoferrin excretion in breast fed babies are in the range of 5.7 to 23.6 mg/day. The babies in this study excreted a mean of 4.5 and 3.67 mg/day for the lactoferrin and lactoferrin plus iron groups.	The addition of lactoferrin had little effect upon the fecal microflora and did not move the pattern of the fecal flora in the direction of the breast fed baby. There was less lactoferrin excreted in the feces than anticipated; it is possible that some of the lactoferrin had been broken down or degraded higher in the gut.	Balmer et al. 1989

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TABLE 21 Fate of Ingested Lactoferrin in the Body			
Study Design	**Results**	**Conclusions**	**Reference**
and the 24 hour specimen collected on day 11 was used to estimate fecal lactoferrin concentration. A blood specimen was taken on day 14.			
Twenty-nine babies were fed from birth either a basic infant formula or the same formula with added bovine lactoferrin (285 mg/L). On the 7th day after birth the babies were given a single dose of ^{58}Fe-labeled bovine lactoferrin or ^{58}Fe-labeled ferric chloride plus ascorbic acid. Iron retention was estimated. Fecal bovine lactoferrin was estimated in a 24-hour stool collection 11 days after birth.	The mean bovine lactoferrin concentration in the 24 hour stool collected on day 11 after birth was 0.55 ± 0.65 mg in infants fed the basic formula and 4.50 ± 1.52 mg in those fed the lactoferrin fortified formula from birth. The babies fed bovine lactoferrin excreted statistically significantly more lactoferrin in their stools than babies fed the basic formula; however, most of the lactoferrin appeared to have been broken down. When a similar assay was performed on stools from 11 day old breast-fed babies, the lactoferrin levels were 8.32 ± 4.28 mg/24 hours, which was significantly higher than babies receiving the lactoferrin formula.	As expected, the babies fed the formula containing added bovine lactoferrin excreted significantly more lactoferrin in their stools than babies fed the basic formula; however, most of the lactoferrin from the basic plus lactoferrin formula appeared to have been broken down.	Fairweather-Tait et al. 1987
Proteins in human milk in human milk fortifiers (Presemp and Enfamil), in preterm formulas (Similac Special Care and Enfalac) and whey protein concentrates with varying degrees of denaturation were digested by duodenal juice from healthy preterm infants, from a 3 year old child, and from adults. Digestion was studied *in vitro*.	Casein was the protein most rapidly degraded in all products. Human and bovine whey proteins were more slowly digested; as much as 68% of human lactoferrin was still immunoreactive after 40 minutes of digestion. Bands of bovine and human milk proteins showed a similar pattern, whether the digestion was performed with juice from the 3-year old or adult.	Results show that whey proteins are slowly hydrolyzed whereas casein is hydrolyzed at a much faster rate.	Lindberg et al. 1998

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TABLE 21 Fate of Ingested Lactoferrin in the Body			
Study Design	**Results**	**Conclusions**	**Reference**
The turnover of ^{125}I-labelled lactoferrin, obtained from breast milk and administered iv, was measured in 10 adults. Simultaneous organ radioactivity counting was performed. Ferrokinetic studies were performed in three adults after the iv injection of [^{59}Fe]lactoferrin.	Lactoferrin was rapidly eliminated from the plasma with a mean fractional catabolic rate of 5.7/day. Assuming the plasma lactoferrin to be in a steady state, the mean synthetic rate was 16.8 mg/day. Of the administered ^{125}I label, 99% was recovered in the urine, as free iodine, within the first 24 hours. In the ^{59}Fe studies, no appreciable activity was found in the urine. Organ radioactivity counting showed that lactoferrin was rapidly taken up by the liver and spleen. the rapid excretion of free ^{125}I suggests catabolism at these sites. In the ^{59}Fe studies, the radioactivity persisted in the liver and spleen for several weeks and was slowly transferred to the bone marrow before appearing in the circulating erythrocytes.	Lactoferrin is found in many human secretions and in neutrophils. The concentration of lactoferrin in human plasma is about 0.2 - 1.5 μg/ml. The plasma lactoferrin concentration is derived from neutrophils; this theory is derived from the correlation of plasma values of lactoferrin with the total neutrophil pool. Lactoferrin is rapidly cleared from the circulation, being avidly sequestered by the liver and spleen. The sequestered lactoferrin is promptly catabolized. The iron, however, is not excreted in the urine, but rather retained and slowly transferred to the bone marrow, where it is subsequently incorporated into developing erythrocytes. These results are based on lactoferrin derived from breast milk and not neutrophils.	Bennett and Kokocinski 1979

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TABLE 21 Fate of Ingested Lactoferrin in the Body			
Study Design	**Results**	**Conclusions**	**Reference**
The rhesus monkey (preterm and infant) was used as an animal model to study the luminal phase of the hydrolysis of proteins in human milk, human milk fortifier, and preterm formula at 6 weeks and 7 months of age.	In six-week old monkeys, the trypsin and elastase activities in the duodenal contents decreased postprandially by 50%, whereas they increased in the 7-month old monkeys. In the preterm and 6 week old monkeys, hydrolysis of human and bovine whey proteins was slow, and in 6-week old monkeys, 30-50% of the proteins could still be detected immunochemically in duodenal contents after 60 minutes. At seven months of age, no or small amounts of lactoferrin could be detected in duodenal contents after 15 minutes.	The low capacity to digest whey proteins in suckling monkeys may depend upon an immaturity of the exocrine pancreas to respond to secretogogues. In the fasting state, the trypsin concentration in duodenal juice from 6-week and 7-month old monkeys is comparable to those found in human infants and children, whereas the elastase activities in 6-week old monkeys are somewhat higher and in 7-month old monkeys, much higher, than in human infants. In conclusion, there is an age-dependent capacity to hydrolyze bovine and human whey proteins in the monkey gut lumen which possibly depends upon an immaturity of the exocrine pancreas of suckling rhesus monkeys to respond to secretogogues.	Lindberg et al. 1997

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TABLE 21 Fate of Ingested Lactoferrin in the Body			
Study Design	**Results**	**Conclusions**	**Reference**
The degradation of bovine lactoferrin was examined in six adult minipigs and three piglets, 21 days old. They were fed 1% by weight lactoferrin in a test meal of 180 g dry matter. Six hours postprandially, the animals were sacrificed and digesta were collected as completely as possible from the different segments of the intestine. The suckling piglets consumed 75 mg lactoferrin in 50 ml fresh sow's milk and intestinal samples were obtained 3 hours postprandially.	Less than 0.25% of the lactoferrin consumed was detected in total digesta of adult pigs. In piglets, however, there was a considerable amount of lactoferrin the distal part of the small intestine (39.2 mg found in the ileum).	Supplementation of the diet of the human newborn with bovine lactoferrin has been reported to result in up to 20% undegraded lactoferrin in the feces. In piglets, a higher amount (52%) was found in the prececal segment of the intestine. This may be the result of a species difference. In addition, it can be expected that a certain percentage of lactoferrin is degraded in the hindgut by microbial activity, which was excluded in this experiment.	Schmitz et al. 1989

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B. Animal Toxicology Studies

The safety of bovine lactoferrin, similar in specifications to the bovine lactoferrin from DMV International, which is the subject of this GRAS determination, was assessed in an acute toxicity study, a 4-week oral toxicity study, a thirteen-week oral toxicity study and an Ames assay.

1. Acute Toxicity Study In Rats

Nishimura (1991) evaluated the acute toxicity of bovine lactoferrin in rats. Male and female Crj:CD (SD) SPF rats were exposed to single oral doses of 1,000 or 2,000 mg/kg bovine lactoferrin (MONL-01) or bovine iron-saturated lactoferrin (MONL-02) via stomach intubation. Controls animals received vehicle alone (2,000 mg/kg water). Animals were observed for mortality, clinical signs, and any changes in general condition during a 14-day observation period following administration. Body weights were measured prior to the study and periodically throughout the observation period. After 14 days, the animals were sacrificed and the organs examined macroscopically for any abnormalities.

Exposure to 1,000 or 2,000 mg/kg MONL-01 and MONL-02 resulted in no deaths or abnormal clinical signs or effects on the general condition of the animals. Lactoferrin had no effect on body weight. There were no significant differences in body weights throughout the study period in treated animals compared to controls. No abnormal gross pathological findings were observed in any organ in the cranial, thoracic, and abdominal cavities.

Lactoferrin exhibited low acute oral toxicity. A single oral dose of 1,000 or 2,000 mg/kg bovine lactoferrin or bovine iron-saturated lactoferrin resulted in no adverse effects or deaths. Based on these results, the lethal dose of lactoferrin exceeds 2,000 mg/kg.

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2. Four-Week Oral Toxicity Study in Rats

The safety of bovine lactoferrin was assessed in a 4-week oral toxicity study in rats (Nishimura 1997). Four-week old male and female Sprague-Dawley rats were exposed by oral intubation to 200, 600, or 2,000 mg/kg/day bovine lactoferrin once daily for 4 weeks (28 days). Animals in the control group received water by the same route of administration. Animals were observed daily for any changes in appearance or behavior. Body weight and food consumption was measured prior to the start of treatment and

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twice weekly every 3 or 4 days prior to dosing. Ophthalmology, urinalysis, hematology, and blood chemistry analyses were conducted in week 4 or at necropsy. Animals in each group were sacrificed on day 29 and observed for external abnormalities. Absolute and relative weights were determined for all organs and tissues in the cephalic, thoracic and abdominal cavities. Organs and tissues of animals in the control group and high-dose group were examined histopathologically.

There were no deaths or changes in the general condition, behavior or appearance of the animals due to administration. Body weight and food consumption were similar in all groups throughout the study; no significant differences were observed between groups. No changes in males or females or significant differences between test and control groups were observed in urinalysis (pH, protein, ketone body, glucose, occult blood, bilirubin, urobilinogen, color, urinary sediments, 24-hour urine volume, osmolarity, sodium, potassium, chloride, or water intake); hematology (red cell count, hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, reticulocyte ratio, platelet count, white blood cell count, differential leukocyte count, prothrombin time, activated partial thromboplastin time, or fibrinogen), or blood chemistry parameters (GOT, GPT, LDH, ALP, total cholesterol, triglycerides, phospholipids, total bilirubin, glucose, blood urea nitrogen, creatinine, sodium, potassium, chloride, calcium, inorganic phosphorus, total protein, A/G ratio or protein fractions).

Absolute and relative body weights did not change significantly throughout the treatment period or differ significantly between groups. Unilateral and/or bilateral persistence of the hyaloid artery in the eye was observed in at least one animal in each group, including the control group. This effect was not considered treatment-related or abnormal because it occurs naturally during the development of the eyeball and disappears with growth. Gross pathological findings observed included excoriation in the neck of 2 males and 1 female in the 200 mg/kg/day group and 1 male in the 600 mg/kg/day group, pneumatosis-like enlargement of the lung in 1 male in the 200 mg/kg/day group, a dark-red spot in the lung of 2 males in the 2,000 mg/kg/day group, and dark-red spots in the glandular stomach in 1 male and 1 female in the control group and 1 female in the 600 mg/kg/day group. Corresponding macroscopic findings were mild ulcer, mild overinflation of the lung, and slight erosion of the stomach, respectively. One 1 female administered 600 mg/kg/day had a fracture of the incisors. These effects are considered incidental since they were not dose-related or consistently observed among the animals. A few microscopic changes were observed in male and female animals that were not considered to be related to treatment. These were cellular infiltration and focal hemorrhage of the lung; erosion in the glandular stomach; cellular

infiltration of the cecum; microgranuloma in the liver; ectopic thymus; tubular basophilia, eosinophilic body in tubular epithelium, and cellular infiltration in interstitium of the kidney; degeneration and necrosis of spermatocyte; decrease of sperm in the epididymus duct; fibrosis in the muscle layer of the esophagus; hyperplasia of ductal epithelium in the sublingual gland; and disarrangement of the retina. These changes, which were slight-to-mild in severity and occurred sporadically in one or two animals, were considered incidental.

Administration of 200, 600, and 2,000 mg/kg/day bovine lactoferrin to male and female rats resulted in no deaths or treatment-related changes in body weight, food consumption, organ weight, ophthalmology, hematology, blood chemistry, urinalysis, or gross pathology and histology examinations. Therefore, the no observed adverse effect level of bovine lactoferrin was estimated to be in excess of 2,000 mg/kg/day.

3. Thirteen-Week Oral Toxicity Study in Rats

As part of a safety assessment of bovine lactoferrin, a 13-week oral toxicity study in rats was conducted (Nishimura 2000). Male and female Sprague-Dawley rats, 4 weeks of age, were exposed to 200, 600, and 2,000 mg/kg/day bovine lactoferrin by oral intubation once daily, seven days a week for 13 weeks. Control animals received vehicle alone. Animals were examined daily for changes in appearance, nutrition condition, or behavior. Body weight and food consumption measurements were taken prior to the start of treatment and twice weekly every 3 or 4 days prior to dosing. Ophthalmology examination and urinalysis (including water consumption), hematology (mean corpuscular volume, red cell count, hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, reticulocyte ratio, platelet count, white blood cell count, differential leukocyte count, prothrombin time, activated partial thromboplastin time, or fibrinogen), and blood chemistry determinations (GOT, GPT, LDH, ALP, total cholesterol, triglycerides, phospholipids, total bilirubin, glucose, blood urea nitrogen, creatinine, sodium, potassium, chloride, calcium, inorganic phosphorus, total protein, A/G ratio or protein fractions) were measured in week 13 (urinalysis was also done at week 6). Animals were sacrificed after 91 days and observed for external abnormalities. Absolute and relative weights were determined for all organs and tissues in the cephalic, thoracic, and abdominal cavities. Histopathological examinations were conducted of all organs and tissues in the control and high-dosed animals, the pancreas of males at all dose levels, and in any animal that died or exhibited macroscopic lesions.

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There were two deaths during the treatment period. One male in the 200 mg/kg/day group died at week 10 without any overt clinical signs of disease. Examination revealed perforation in the esophagus and hydrothorax with food. The death was attributed to an error in intubation. One female in the 2,000 mg/kg/day group exhibited swelling of the subcutis at week 12 and died at week 13. At necropsy, enlargement of the lymph nodes, thymus, spleen, and liver and white spots in the kidney were observed. Upon histologic examination, malignant lymphoma and tumor cells in the brain, harderian gland, heart, lung, pituitary, adrenal, ileum, cecum, ovary, uterus and bone marrow were observed. Slight extramedullary hematopoiesis was seen in the adrenal gland. Death of the animal was attributed to the presence of malignant lymphoma.

No abnormal clinical signs due to administration of bovine lactoferrin were seen in surviving animals. However, a few clinical signs deemed incidental because of their sporadic occurrence were observed. These were subcutaneous mass in the axillary mass accompanied by hemorrhage and paleness of skin in 1 male in the control group, fracture of incisors in 1 female in the control group, and excoriation in the neck in 1 female in the 200 and 600 mg/kg/day groups and 2 females in the 2,000 mg/kg/day group. One female in the 2,000 mg/kg/day group had decreased spontaneous movement and oligopnea. Perforation in the esophagus, hydrothorax, and dark-reddening of the lung was observed at necropsy suggesting that the effects were due to an error in administering the compound.

No significant differences were observed in body weight or food consumption between groups during the treatment period. No ophthalmologic abnormalities were observed in any animal. Hematology and blood chemistry determinations were not significantly different between the study groups. At week 6, no significant changes in urinalysis parameters were observed in animals administered 200 mg/kg/day; at week 13, significant increases in urine volume and daily excretion of sodium, potassium, and chloride in males occurred. These changes, however, were not dose-related. In the 600 mg/kg/day group, significant decreases in daily excretion of sodium were observed in males at week 6; however, no significant changes were observed in any urinalysis parameter at this dose at week 13. In the 2,000 mg/kg/day group, pH and daily excretion of sodium were reduced in males at week 6. At week 13, the reduction in pH was seen in male and females and there was no change observed in sodium excretion. The change in urinary pH is not thought to be of toxicological significance since there was only a slight degree of change and there were no significant changes in any other urinary parameters such as volume and content of electrolytes. In addition, there were no corresponding histological findings in the kidney or blood chemistry values.

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No changes in absolute or relative organ weights were observed in males or females administered 200 or 600 mg/kg/day. At the highest dose administered, significant decreases in absolute and relative thyroid weights compared to controls were observed in females ($p<0.05$). These changes were within the normal histological background range. In addition, no corresponding findings were observed upon histopathological examination.

Gross pathology findings in a few animals and histopathologic changes in several organs were observed. These findings were not consistently observed among animals and were considered incidental. No histopathological findings were considered to be treatment related, but were judged incidental in view of their occurrence and the nature of the lesions. Slight or mild islet fibrosis of islet acinar cells were observed in 3 males in the control group and 6 males each in the 200, 600, and 2,000 mg/kg/day groups. Although the incidence and severity of the finding in each treated group was slightly higher than that of the controls, these findings were not considered to be treatment-related. No morphological differences in the fibrosis of islets between the control and treated groups were observed and the distribution of lesions in animals in the treated groups were limited to the same small section of tissue as in the control animals.

Bovine lactoferrin administered by oral intubation to rats for 13 weeks did not result in significant treatment-related changes in the appearance, general condition, body weight, food consumption, ophthalmology, hematology, blood chemistry, gross pathology, or histopathology of the animals. Thus, under the conditions of this study, the no-observed-adverse-effect level of bovine lactoferrin was estimated to be in excess of 2,000 mg/kg/day.

4. Ames Assay

The genotoxic potential of bovine lactoferrin was examined in the reverse mutation assay (Kawai and Tanaka 1997). The assay was performed using *Salmonella typhimurium* strains TA100, TA1535, TA98, and TA1537 and *Escherichia coli* strain WP2*uvr*A, with and without metabolic activation. Metabolic activation was provided by an Aroclor-induced rat liver microsome fraction (S9 mix). The tester strains were exposed to lactoferrin via the preincubation method. Lactoferrin was tested at six concentrations: 160, 320, 630, 1,250, 2,500, and 5,000 μg per plate, based on the results of a dose range finding test. Physiological saline was used as the vehicle control. In the absence of metabolic activation, the positive controls were 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide for tester strains TA100, TA98, and WP2*uvr*A, sodium azide for tester strain TA1535, and 9-aminoacridine for tester strain TA1537. In the presence of metabolic

activation, the positive controls were benzo(a)pyrene for tester strains TA100, TA98, and TA1537 and 9-aminoacridine for tester strains TA1535 and WP2*uvr*A. Lactoferrin was evaluated in duplicate for each concentration in the presence or absence of metabolic activation.

No precipitation or crystallization was observed at any lactoferrin concentration. The positive controls for the tester strains yielded the expected number of revertants per plate of at least a 2-fold increase in the number of revertants relative to the vehicle control. For the test article to be classified as producing a positive result, there had to be a proportional increase in the number of revertant colonies relative to the increase in the concentration of lactoferrin and the ratio of the number of revertant colonies to that of the control group had to be at least 2.0. For all concentrations of lactoferrin in all tester strains, with or without metabolic activation, the ratio of the number of revertant colonies to that of the control group was 1.4 or lower. A second assay produced similar results.

Lactoferrin did not cause a positive response with any of the tester strains in the presence or absence of S9 activation at concentrations up to 5,000 μg/plate. Thus, under the conditions of this study, lactoferrin was not found to be genotoxic in the *Salmonella typhimurium* reverse mutation assay or *Escherichia coli*/mammalian microsome reverse mutation assay.

C. Allergenicity

Allergic reactions to foods and dietary components of food are common. While the degree of sensitivity to food allergens varies from individual to individual, even trace amounts of an offending food allergen may trigger a reaction. Symptoms can include hives, vomiting, diarrhea, and respiratory distress. In some cases, just one bite of food can bring on anaphylaxis, and in extreme cases, it can cause death. Cow's milk, egg white, peanuts, shellfish, and soybeans are among the known food allergens.

Cow's milk allergy is particularly prevalent among infants and children. There are various proteins in milk known to be food allergens, including β-lactoglobulin, α-lactalbumin, caseins, bovine immunoglobulins, and bovine serum albumin. IgE antibodies to several other minor milk proteins -including lactoferrin- have been identified in a few patients (Baldo 1984). Lactoferrin protein has also been identified as eliciting allergic reactions in several animal models tested. Individuals, especially children, who are allergic to cow's milk should be aware of the allergenicity to lactoferrin. The following studies demonstrate the potential of lactoferrin to induce allergic reaction.

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Host et al. (1992) analyzed specific IgE to bovine milk proteins including lactoferrin by crossed radioimunoelectrophoresis (CRIE). The distribution of elevated specific IgE antibodies

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to several cow milk proteins showed a high frequency of antibodies to bovine se-albumin and less frequency to IgG, alfa-lactoalbumin and lactoferrin in the serum samples from infants with cow milk allergy. At 12 months of age the frequency of IgE to lactoferrin increased in infants with IgE mediated cows milk allergy from 0 in 18 and 17 (cord blood and 6 months of age, respectively) to 5 in 20 infants following a challenge with bovine milk.

Atkinson and Miller (1994) used serum from Brown Norway (BN) rats fed various levels of skimmed milk (SSM) to profile the milk protein antibody responses to the antigens present in SSM. The rats produced both IgG and reaginic antigen-specific response against lactoferrin. The reaginic response to lactoferrin was similar to alpha-casein and higher than that produced against bovine serum albumin, the lactoglobulins, and β- or κ-casein.

Miller et al. (1998) investigated the allergenicity of lactoferrin in studies using Brown Norway (BN) rats. Using parenteral sensitization regimens, the authors reported the highest dose that did not induce reaginic antibody production for lactoferrin (0.01 μg), ovalbumin (0.1 μg) and bovine serum albumin (1 μg). Therefore, the comparative allergenic potential of these proteins was lactoferrin>ovalbumin>bovine serum albumin. Semiskimmed milk (SSM) was found to be less allergenic than ovalbumin as the total dose of antigen required to induce sensitization to SSM was 20-fold greater than that required for ovalbumin.

Miller et al. (1998) also demonstrated that BN rats sensitized parenterally with 500 μg SSM on days 0 and 7 developed reaginic IgE responses to a range of milk proteins, including lactoferrin. Under these conditions, lactoferrin was found to be as allergenic as the caseins and β-lactoglobulin in the BN rat.

Debbabi et al. (1998) investigated the nature of the immune responses induced by repeated oral administration of bovine milk lactoferrin in mice and detected anti-lactoferrin IgA and IgG in the intestinal fluid and serum of mice fed lactoferrin daily for four weeks. Total immunoglobulins were also higher in the intestinal fluid in lactoferrin-fed mice than in the control group. Debbabi et al. suggest that lactoferrin could act as an immunostimulating factor on the mucosal immune system and that activation of the mucosal immune system is dependent on the ability of lactoferrin to bind to the intestinal mucosa.

D. Human Exposure

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Bovine lactoferrin has been consumed by humans for thousands of years as a naturally occurring protein found in milk. Consumption of bovine lactoferrin through dairy sources has been estimated to be 73, 75, and 50 mg/day at the 90th percentile of intake for children, teens and adults, respectively. In addition to dairy sources, lactoferrin is currently marketed in the U.S. as a dietary supplement. Consumption of lactoferrin from this source ranges from 10 to 1,200 mg/day.

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Infants who are breast-fed consume human lactoferrin which is a natural constituent of human milk. Human milk contains from 1 to 4.2 mg/ml, resulting in a daily consumption of 1 to 4.2 g/day, assuming a daily consumption of 1 liter. As a comparison, infants who consume infant formula derived from cow protein only consume up to 245 mg/day of bovine lactoferrin, derived from the whey protein component. There are several infant formulas, however, currently sold in countries outside the U.S. that contain supplemental lactoferrin. The supplemental lactoferrin adds up to 107 mg/day in addition to the maximum of 245 mg/day derived from whey protein. Therefore, infants consuming these formulas may consume up to 352 mg/day bovine lactoferrin.

E. Human Clinical Trials

Bovine lactoferrin was ingested by both infants and adults in numerous clinical trials that were designed to look at effects on either iron absorption or modulation of microflora or infection. In studies of infants, dose levels used ranged from 1.4 mg/day (0.3 mg/kg/day) to 2.9 g/day (1.0 g/kg/day) and study durations were from 11 days to 5 months. In studies of adults, dose levels used ranged from 100 mg/day (1.7 mg/kg/day) to 3.6 g/day (60 mg/kg/day) and study durations varied from a single dose in one study to 8 weeks. These studies are summarized in Table 22.

Despite the fact that the studies were not designed specifically to look for issues of tolerance to ingestion of lactoferrin, no adverse health effects as a result of lactoferrin intake are reported in any of the studies. There are also no indications of subjects discontinuing due to adverse reactions. These data are also helpful, from the standpoint of assessing safety and tolerance to infants, due to the number of studies that were conducted using long durations. Of the seven studies looking at effects of lactoferrin in infants, four were conducted using supplemental lactoferrin feeding for periods of 3-5 months, the time during which infants are utilizing formula as a sole source of nutrition. In the studies of adult subjects, not only healthy individuals, but subjects with leukemia, chronic hepatitis and tinea pedis were given bovine lactoferrin without any reported adverse effects.

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TABLE 22 Human Studies of Lactoferrin			
Study Design	**Objective**	**Results**	**Reference**
Iron balance studies were performed in 16 term infants from their 3rd until their 17th week of life. Seven infants were fed an adapted infant formula supplemented with bovine lactoferrin (100 mg/100 ml) and nine received the same formula without lactoferrin. Assuming a mean intake of formula of 1,031 ml/day or 194 ml/kg/day for infants 0-4 months old and a bodyweight of 5.3 kg; the dose of ingested lactoferrin was 1.0 g/day or 194 mg/kg/day.	Iron balance studies were comprised of five periods with an interval of 3 to 4 weeks, each consisting of three 24-hour collections of milk and stool samples.	Iron retention in the lactoferrin supplemented group was 63 µg/kg/day and in the unsupplemented group it was 43 µg/kg/day. Mean percentage retention of iron in the supplemented group was 36%, in the unsupplemented group 28%. Although iron availability was slightly better in the supplemented group, this difference was not significant. A further comparison with previous work on ingestion of human milk indicated that although iron bioavailability from human milk was significantly better than both formula studies; the absolute iron retention in the breast fed infants (32 µg/kg/day) was lower than in the formula groups. No adverse health effects of lactoferrin consumption were reported.	Schulz-Lell et al. 1991
Infant formula supplemented with various amounts of bovine lactoferrin (10 or 100 mg lactoferrin/100 ml formula) was given to two groups (n=14 for each group) of infants for 150 days starting from birth. These infants were compared with infants receiving unsupplemented formula (n=14) and breast-fed babies (n=10). Assuming a mean intake of formula of 1,031 ml/day or 194 ml/kg/day for infants 0-4 months old and a bodyweight of 5.3 kg; the dose of ingested lactoferrin was 1.0 g/day or 194 mg/kg/day at a concentration of 100 mg/100 ml and 0.1 g/day or 19.4 mg/kg/day at a concentration of 10 mg/100 ml.	Effects of the diets on levels of hemoglobin, hematocrit, serum iron, ferritin and zinc were examined for the 150-day study period.	At birth, concentrations of iron, hemoglobin, hematocrit, and zinc were comparable in all four feeding groups. During the first three months of life, hemoglobin, hematocrit and iron serum levels were not substantially different in the four feeding groups. No difference in serum zinc values was demonstrated between groups at any time during the study period. Serum ferritin was measured to provide a way to calculate the amount of total body iron stores. Serum ferritin levels increased from day 0 to 7 in all groups and persisted at these levels at day 30. The levels then decreased (day 90 and 150). At day 30, ferritin was significantly higher in the human milk group compared to all the formula-fed groups. At day 90, significantly higher ferritin values were observed in the human milk group compared to the unsupplemented formula group, but these levels were not significantly different than those from the lactoferrin supplemented group. At day 150, lactoferrin (100 mg/100 ml) supplemented formula-fed infants had serum ferritin levels significantly higher than the unsupplemented formula group. No adverse health effects of lactoferrin consumption were reported.	Chierici et al. 1992

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TABLE 22 Human Studies of Lactoferrin			
Study Design	**Objective**	**Results**	**Reference**
Formula was given to babies for fourteen days. Formulas used were (a) no added iron and no added lactoferrin (n=20); (b) no iron but added lactoferrin (n=18); (c) added iron and lactoferrin (n=20). Lactoferrin was added at 2.8 g/L formula. Anthropometric measurements were made on day 1. Feces were collected on days 4, 11 and 14. Specimens collected on days 4 and 14 were used to measure the bacterial flora and the 24-hour specimen collected on day 11 was used to estimate fecal lactoferrin concentration. A blood specimen was taken on day 14. Babies weighed approximately 3 kg. Using this weight, and the default value of mean intake of formula of 1,031 ml/day, intake of lactoferrin was approximately 2.9 g/day or 1.0 g/kg/day.	The objective of the study was to determine the effects of lactoferrin on the fecal flora.	The addition of lactoferrin had little effect on the fecal microflora and did not move the pattern of the fecal flora in the direction of the breast-fed baby. Lactoferrin excretion in the feces was greater in the lactoferrin-fed babies than in those fed the basic formula, however, observed excretion was far below that expected. Reports of lactoferrin excretion in breast-fed babies are in the range of 5.7 to 23.6 mg/day. The babies in this study excreted a mean of 4.5 and 3.67 mg/day for the lactoferrin and lactoferrin plus iron groups. No adverse health effects of lactoferrin consumption were reported.	Balmer et al. 1989
Four infant feeding groups were studied. Fifty-one infants finished the study; an additional four infants provided two samples during the study period. The subjects were fed the following diets: group A, breast-feeding (n=12); group B, formula feeding (n=14) infants; group C, experimental formula supplemented with 10 mg/100 ml lactoferrin (n=15); and group D, experimental formula supplemented with 100 mg/100 ml lactoferrin (n=14). Study duration	The objective of the study was to investigate the development of the fecal flora in infants given lactoferrin supplemented formula.	Breast-fed infants developed a flora rich in Bifidobacteria. Facultative anaerobes were ubiquitous, but in relatively small numbers within the diet group. Other obligate anaerobes, such as *Clostridium* sp. and *Bacteroides* sp. were rarely isolated. Standard formula produced a flora rich in bifidobacteria, but the growth of facultative organisms was not suppressed by this diet. *Clostridium* sp. and *Bacteroides* sp. were more common in this feeding group. The addition of 10 mg lactoferrin/100 ml formula produced a flora similar to that of the standard formula fed babies. Lactoferrin at 100 mg/100 ml was able to establish a "bifid flora" in half of the babies given this formula, but only at age three months. *Clostridium* sp. and *Bacteroides* sp. were common isolates	Roberts et al. 1992

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TABLE 22 Human Studies of Lactoferrin			
Study Design	**Objective**	**Results**	**Reference**
was 3 months. Assuming a mean intake of formula of 1,031 ml/day or 194 ml/kg/day for infants 0-4 months old and a bodyweight of 5.3 kg; the dose of ingested lactoferrin was 1.0 g/day or 194 mg/kg/day at a concentration of 100 mg/100 ml and 0.1 g/day or 19.4 mg/kg/day at a concentration of 10 mg/100 ml.		from babies receiving both the lactoferrin diets. No adverse health effects of lactoferrin consumption were reported. The formula was supplemented with lactoferrin at levels that are relatively low compared with human milk (200-600 mg/100 ml milk). This may have contributed to the lack of effect of lactoferrin on the fecal flora. In addition, the fact that the lactoferrin was 20% saturated with iron may have negated or weakened its effect.	
Iron absorption was measured from human milk in eight breast-fed infants with the native level of lactoferrin present and after removal of greater than 97% of the lactoferrin. The infants ranged in age from 2-10 months (mean age = 5 mo.). The study had a randomized, cross-over design and Fe absorption was measured by stable isotope technique, using ^{58}Fe as a label, with Fe absorption measured by analysis of the incorporation of ^{58}Fe into RBCs 14 days after administration.	The study was designed to investigate the influence of lactoferrin on iron absorption from human milk in infants.	Total volume of labeled milk varied between 700-1,000 g/administration, but was held constant between administrations for each infant. Absorption of Fe was found to be significantly higher when lactoferrin-free breast milk was fed, as compared to breast milk. The mean absorption was 11.8% for breast milk and 19.8% for lactoferrin-free breast milk. In the youngest infant studied (2 months old), Fe absorption was higher from human milk than from the lactoferrin-free milk. No adverse events were reported.	Davidsson et al., 1994
Twenty-nine babies were fed from birth either a basic infant formula or the same formula with added bovine lactoferrin (285 mg/L). On the 7th day after birth the babies were given a single dose of ^{58}Fe-labeled bovine lactoferrin or ^{58}Fe-labeled ferric chloride plus ascorbic acid. Iron retention was estimated by measuring the unabsorbed ^{58}Fe excreted in the feces during the 3 days following	The study was conducted to investigate the effect of lactoferrin on iron absorption in infants. The extent of lactoferrin broken down by digestive enzymes and microorganisms was also investigated.	Mean percent Fe retention from ferric chloride was 44.4 % and 46.2 % from lactoferrin. There was no significant difference between the two iron sources, nor did the previous formula (basic vs basic plus lactoferrin) have any effect on iron retention from either ferric chloride or lactoferrin. As expected, the babies fed the formula containing added bovine lactoferrin excreted significantly more lactoferrin in their stools than babies fed the basic formula; however, most of the lactoferrin from the basic plus lactoferrin formula appeared to have been broken down.	Fairweather-Tait et al, 1987

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TABLE 22 Human Studies of Lactoferrin			
Study Design	**Objective**	**Results**	**Reference**
administration. Fecal bovine lactoferrin was estimated in a 24-hour stool collection 11 days after birth. Mean body weight was 3.1 kg. Assuming a formula consumption of 1,031 ml/day, the lactoferrin dose ingested was approximately 294 mg/day or 95 mg/kg/day.		No adverse events were reported.	
Six groups of healthy, term infants 6 wks of age were fed breast milk or a cow's milk-based formula (with varying amounts of iron, selenium, copper, and in one group lactoferrin) until the infants were 6 months old. Groups B and C were fed 4 mg/L iron, 10 μg/L selenium. The iron source for Group B was $FeSO_4$ while Group C received 1.4 mg/L as bovine lactoferrin (35% of iron) and 2.6 mg/L as $FeSO_4$. There were 10 infants per group. Hematological indices analyzed included hemoglobin, serum iron, transferrin, serum ferritin and serum transferrin receptor). Also measured were BUN, height, weight, serum copper, ceruloplasmin, serum zinc, and α-macroglobulin. Assuming a mean intake of formula of 1,031 ml/day or 194 ml/kg/day for infants 0-4 months old and a bodyweight of 5.3 kg; the dose of ingested lactoferrin was 1.4 mg/day or 0.3 mg/kg/day at a concentration of 1.4 mg/L.	This study investigated the effect of different levels of iron, copper, and selenium fortification of infant formula on the mineral status of infants. Specifically, selenium, iron, zinc, and copper status were evaluated.	BUN values were significantly lower in breast-fed infants than in formula-fed infants at 6 weeks of age. At 6 months, these differences were smaller but still significant. There were no significant differences in birth weight, weight or height at 6 weeks or 6 months of age among the groups. No significant differences were observed in the hematological parameters when comparing Group C receiving lactoferrin with the group receiving the same amount of iron (4 mg/l) as ferrous sulfate (Group B). There was no significant effect of copper supplementation of formula on plasma copper or ceruloplasmin levels when comparing infants fed a formula without supplementary copper and the identical formula with 0.4 mg/l of supplemental copper. Supplementation with selenium as selenite at a level of 10 μg/l resulted in a similar selenium status in formula-fed infants as that of breast-fed infants. Plasma zinc levels were not significantly different between groups. No adverse events from the intake of lactoferrin were reported.	Lönnerdal & Hernell, 1994

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TABLE 22 Human Studies of Lactoferrin			
Study Design	**Objective**	**Results**	**Reference**
Ten healthy male volunteers were given bovine lactoferrin (2 g/person/day or 33 mg/kg/day assuming a 60 kg body weight) for 4 weeks. Blood samples were drawn before, during and after administration of lactoferrin.	Phagocytic activity and superoxide production activity of polymorphonuclear leukocytes (PMN) were evaluated. Expression levels of CD11b, CD16 and CD56 molecules on leukocytes were quantified using flow cytometry.	The phagocytic activity or superoxide production activity of PMN or the proportions of CD11b+, CD16+ and CD56+ in lymphocytes were increased during the period of lactoferrin administration. The effects on the immune system were different in individual cases. Lactoferrin may influence primary activation of the host defense system. No adverse health effects of lactoferrin consumption were reported.	Yamauchi et al. 1998
Five neutropenic patients receiving chemotherapy for acute myelogenous leukemia were given lactoferrin (4 x 200 mg/day, for a total of 800 mg/day or 13 mg/kg/day assuming a body weight of 60 kg, p.o., days 1 through 10 of neutropenia). Nine patients who fulfilled the admission criteria but received no prophylaxis except amphotericin B tablets for the prevention of oral candidiasis served as controls.	To determine if lactoferrin, after enteral administration, is able to prevent, postpone or attenuate enterogenic infection in patients with chemotherapy-induced neutropenia.	Lactoferrin treatment did not significantly prolong the infection-free interval (4.4 ± 2.6 days versus 3 ± 1.9 days in controls). It did, however, significantly shorten the duration of the first infection (3.4 ± 2.7 days versus 7.7 ± 3.5 days in controls). Bacteremias with *Enterobacteriaceae* were observed in only two of five lactoferrin-treated patients compared to six of nine controls. No adverse health effects of lactoferrin consumption were reported.	Trümpler et al. 1989
Eleven patients with chronic hepatitis C received an 8-week course of bovine lactoferrin (1.8 or 3.6 g/day corresponding to 30 or 60 mg/kg/day, respectively, assuming a body weight of 60 kg).	The objective of this study was to test the hypothesis that lactoferrin inhibits hepatitis C virus (HCV) viremia in patients with chronic hepatitis C.	At the end of lactoferrin treatment a decrease in serum alanine transaminase and HCV RNA concentrations was apparent in 3 (75%) of 4 patients with low pretreatment serum concentrations of HCV RNA. However, 7 patients with high pretreatment concentrations showed no significant changes in these indices.	Tanaka et al. 1999
Either 600 or 2,000 mg lactoferrin/day (10 or 33 mg/kg/day, assuming a body weight of 60 kg) was administered orally for 8 weeks to 37 adults who were judged to have mild or moderate tinea pedis. Controls received a placebo.	The study was conducted to evaluate the effectiveness of lactoferrin on the treatment of tinea pedis.	In the analysis of all subjects, dermatological symptoms scores in all groups decreased, and the differences were not statistically significant comparing all three groups. However, in the analysis limited to subjects with moderate vesicular or interdigital tinea pedis, dermatological symptoms scores in the lactoferrin treated groups decreased significantly as compared to the placebo group. There were no adverse events for any of the thirty-seven	Yamauchi et al. unpublished

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TABLE 22 Human Studies of Lactoferrin			
Study Design	**Objective**	**Results**	**Reference**
		subjects and no subject withdrew because of an adverse event.	
Eight healthy iron-sufficient adult subjects were fed a single dose of 100 ml of a test milk (human milk, simulated human milk, simulated human milk containing 100 mg lactoferrin per 100 ml milk (total dose of 100 mg lactoferrin or 1.7 mg/kg assuming a body weight of 60 kg), and two commercial infant formulas). Feedings were conducted at intervals over two weeks. The iron content of each of these milks was 66, 70, 210, 1250, and 1,250 ug/100 ml milk, respectively. Blood samples were obtained 14 days following sample ingestion, just prior to ingestion of the next milk sample to be studied. Incorporation of iron into RBCs was determined.	The study was designed to compare the absorption of iron from several proprietary formulas with that from human milk in an attempt to identify the factor, or factors, responsible for the enhanced iron availability of human milk.	The percent iron incorporation was greatest from the human milk (15.4%) compared to simulated human milk (9%), simulated human milk with added lactoferrin (4.7%), SMA (3.08%), and Similac (2.6%). Actual iron incorporation was greatest in the iron-fortified formulas (approx 0.038 mg/100 ml) followed by human milk (0.010 mg/100 ml), and simulated human milk (0.006 mg/100ml). The addition of lactoferrin to the simulated human milk increased actual iron incorporation to 0.010 mg/100 ml. No adverse events were reported.	McMillan et al. 1977

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Pages 000330 - 000339 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

APPENDIX B:

Safety Information Sheets for
Processing Chemicals and Processing Aids

000340

Product code : 11055
Product name : Raw milk
Date : 12-22-1997
Edition # : 02
Status : Final

Analysis code	Reference	Value		
0029	Total solids	typ. 13.4	%	4
0265	Lactose(Luff)	typ. 4.70	%	4
0200	Protein (Nx6.38)	typ. 3.50	%	4
0347	Fat (RG)	typ. 4.50	%	4
0006	ash 550c	typ. 0.8	%	4
0041	odor	conform standard. free of unusual odors		
0097	temperature	max 6°C		
0399	acidity	16-20 N		
0285	Lactate(in 100g ffts)	max. 30mg/100g		
0116	Freezing point	max. – 0.515°C		
0721	Growth inhibitors	negative (≤0.003 IU/ml)		
0120	Scorched particles	disc 1 in 1000ml		
0894	pH fat in 100g	max. 0.8 mmol		
0547	Nitrate, in ffts	max. 12mg/kg		
0321	Radioactivity	max. 2 Bq/kg		
0153	Aflatoxine M-1	max. 50 ppt		
	Dioxine	max. 6 pg/kg milk fat		
0700	Plate count 30°C	max. 1000000 cfu/g		
0709	Thermoresistant bacteria63,5°C	max. 5000 cfu/g		
0704	Aerobic spores 30°C	max. 1000 cfu/g		
0760	Bacillus Cereus	max. 1 cfu/g		

Remarks:

1 These specifications apply at arrival in the plant.
2 Products of this specification have to comply with the demands written in the EEG food law. (Beneluxbesluit 02.15.91. EEG richtlijn 92/46 /EEC. Nederlandse warenwet. EEG richtlijn 86/363). also the definition heavy metals, pesticides and PCB's.
3 Storage max. 36 hours at 6°C.
4 No inspection criteria. only related to result counts.
5 Raw milk is milk given only by cows. You can't add other milk elements. milk powder. lactose, buttermilk, whey elements. milk permeate. ultrafiltrate or bactofugate.

000341

Product code : 36026
Product name : Sodium chloride
Date : 09-18-1998
Edtion nr. : 04
Status : in progress

Description : Dry. sifted consumptive salt. (buttersalt)
White crystal powder. Food grade
Contains max. 10ppm anti-coagulant (E535/E536)

Analysis code	Reference	Value
0030	total solids	min 99.8%
0529	sodium chloride	min 99.7%
0884	fineness ⌀125um	max. 14.0%
0142	fineness ⌀250um	min. 90.0%
0148	fineness ⌀1000um	100%
0098	bulk density	1.200-1.400 g/l
0120	scorched pant.	Disc 1
0051	color	conform standard. white
0095	odor	conform standard. salt
0419	arsenic	max. 3mg/kg
0581	heavy metals	max. 5mg/kg
1162	sulfate	max. 0.1%
0425	calcium	max. 15mg/kg
0494	copper	max. 0.1mg/kg
0570	Iron	max. 2.0mg/kg
0510	magnesium	max. 1.0mg/kg

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CENTRALE AANKOOP

FNZ

VEILIGHEIDS- INFORMATIEBLAD (volgens EG-richtlijn 93/112/EEG)
Boterzout FFP

1. IDENTIFICATIE

Productnaam	: Boterzout FFP
Gebruikelijke chemische naam	: Sodium chloride (NaCl)
Leverancier	: BV Centrale Aankoop FNZ
Telefoonnummer voor	: Nationaal Vergiftigingen Informatie Centrum, noodgevallen tel: (030) 2 74 88 88 (Uitsluitend voor een behandelend arts bij vergiftiging)

2. SAMENSTELLING EN INFORMATIE OVER DE BESTANDDELEN

Informatie over gevaarlijke componenten

Chemische naam	*CASnr.*	*% w/w*	*EG-nr.*	*Symbool*	*R-zinnen*	*Annex-1 nr*
Sodium chloride	7647-14-5	± 100	2315983	Geen	Geen	Geen

3. GEVARENIDENTIFICATIE

Niet als gevaarlijk geclassificeerd volgens de EG-richtlijn voor gevaarlijke stoffen en de EG-richtlijn voor gevaarlijke preparaten.

4. EERSTE HULP MAATREGELEN

Effecten en symptonen	: Irriterend voor de ogen.
Bij inslikken	: Wek na overmatige opname braken op.
Bij inhalatie	: Niet van toepassing.
Huidcontact	: Spoelen met water.
Oogcontact	: Spoel grondig met veel water. Vraag geneeskundig advies indien irritatie optreedt.
Advies aan de arts	: Geen specifieke aanbevelingen.

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KPMG ISO 9002

B.V. Centrale Aankoop FNZ, Graafsingel 2Z, 6921 RT Duiven, Postbus 93, 6920 AB Duiven
Telefoon 026 - 318 67 11, Fax algemeen 026 - 311 91 95, Handelsregister nr. 87792 te Arnhem,
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Boterzout FFP

5. BRANDBESTRIJDINGSMAATREGELEN

Blusmiddelen Te gebruiken	: Alle media.
Niet te gebruiken	: Geen.
Ongebruikelijke brand/ explosie risico's	: In geval van brand en/of explosie inademen van rook vermijden.
Gevaarlijke thermische ontleding- en verbrandingsproducten	: Ontwikkelt in een brandende omgeving vergiftige dampen (chlorine).

6. MAATREGELEN BIJ ACCIDENTEEL VRIJKOMEN VAN DE STOF OF HET PREPARAAT

Persoonlijke voorzorgsmaatregelen	: Geen specifieke aanbevelingen.
Milieuvoorzorgsmaatregelen	: Verzamel zoveel mogelijk in een schone container, om af te voeren of (beter) om te hergebruiken.
Reinigingsmethoden	: Veeg op en doe in een container om te worden afgevoerd. Spoel het restant weg met ruim water.

7. HANTERING EN OPSLAG

Hantering	: Geen specifieke aanbevelingen.
Opslag	: Bewaar op een droge plek (max. relatieve vochtigheid = 75%)
Brand- en explosiepreventie	: Niet van toepassing.

8. MAATREGELEN TER BEHEERSING VAN BLOOTSTELLING/PERSOONLIJKE BESCHERMING

000344

Grenswaarden beroepsmatige

000344

Datum van uitgifte : 12 oktober 1993 Printdatum: 10-08-99

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Boterzout FFP

blootstelling	: Er is geen blootstellingslimiet vastgelegd.

Persoonlijke beschermingsmiddelen

Ademhalingstelsel	: Niet relevant.
Huid en lichaam	: Niet relevant.
Handen	: Niet relevant.
Ogen	: Veiligheidsbril.

9. FYSISCHE EN CHEMISCHE EIGENSCHAPPEN

Verschijningsvorm	: Kristal
Kleur	: Wit
Geur	: Geen
Kookpunt/-traject	: 1465°C
Smeltpunt/-traject	: 801°C
pH	: 7,5 (18°C)
Oplosbaarheid in water	: 310 g/l (18°C)
Oplosbaarheid in ander oplosmiddelen	: Oplosbaar in glycerol.
Dichtheid	: 2170 kg/m^3 (20°C)
Vlampunt	: Niet van toepassing
Vlambaarheid	: Niet ontvlambaar
Zelfontbrandingstemperatuur	: Niet van toepassing
Explosie-grenzen	: Niet van toepassing
Explosie eigenschappen	: Geen
Oxyderende eigenschappen	: Niet van toepassing
Stortdichtheid	: ± 1250 kg/m^3 (Broxetten: niet van toepassing)
Verdelingscoëfficiënt n-octanol/water	: log Pow = -3.0 (berekend)
Relatieve dampdichtheid	: Niet van toepassing (lucht = 1)
Viscositeit	: Niet van toepassing
Dampdruk	: Niet van toepassing
Stabiliteit	: Bestendig onder aanbevolen bewaar- en hanteercondities (zie sectie 7).
Te vermijden omstandigheden	: Reageert heftig met BrF_3.
Te vermijden substanties	: Metalen (behalve roestvrijstaal).
Gevaarlijke ontledings-producten	: Indien betrokken bij brand (Cl- en Na_2O).

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11. TOXICOLOGISCHE INFORMATIE

Chemische naam : Sodium chloride.

Acute toxiciteit

Via de mond LD50 : Rat: 3000 mg/kg.

Prikkeling

Contact met de ogen : Irriterend voor de ogen.

12. ECOLOGISCHE INFORMATIE

Geen gegevens beschikbaar.

13. INSTRUCTIES VOOR AFVALVERWERKING

Product : Geen specifieke aanbevelingen.

Gecontamineerde verpakking : Geen specifieke aanbevelingen.

14. INFORMATIE MET BETREKKING TOT HET VERVOER

UN N° : Geen.

Land- Weg/Spoorwegen
ARD/RID
klasse : Niet beperkt.
nummer : Niet relevant.
Gevaarsaanduiding nr. : Niet relevant.
TREM-kaart : Niet relevant.
Proper shipping name : Niet relevant.
Stofaanduiding nr. : Niet relevant.

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Zee
IMO/IMDG

klasse	: Niet beperkt.
code	: Niet beperkt.
Verpakkingsgroep	: Niet relevant.
EMS	: Niet relevant.
MFAG	: Niet relevant.
Zeevervuilende stof	: Neen.
Proper shipping name	: Niet relevant.

Lucht

ICAO/IATA/DGR klasse	: Niet beperkt.
Verpakkingsgroep	: Niet relevant.
Proper shipping name	: Niet relevant.

15. WETTELIJK VERPLICHTE INFORMATIE

EG regelgeving

Chemische beschrijving	: Sodiumchloride (NaCl)
Classificatie volgens	: EG-richtlijnen.
Gevarensymbool	: Geen.
Waarschuwingszinnen	: Geen.
Veiligheidszinnen	: Geen.
Wassergefährdungsklasse (WKG) (Duitsland)	: 0 (kommision Bewertung Wassergefährdender Stoffe)

16. OVERIGE INFORMATIE

Waarschuwing	: Bovenstaande gegevens zijn met de grootst mogelijke zorg verzameld uit bestaande literatuur. BV Centrale Aankoop FNZ aanvaardt geen enkele aansprakelijkheid voor schade, van welke aard of omvang ook, die uit het gebruik van deze gegevens zou kunnen voortvloeien.

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Telefoon 026 - 318 67 11, Fax algemeen 026 - 311 91 95, Handelsregister nr. 87792 te Arnhem,
Postbank 4221205, Rabobank 38.39.49.211, BTW nr.: NL 8041.57.315 B01

VEILIGHEIDS- INFORMATIEBLAD (volgens EG-richtlijn 93/112/EEG)
Boterzout FFP

Historie

Datum 1e uitgifte	: 12 oktober 1993	
Datum uitgifte	: 12 oktober 1993	
Versie	: 1	
SDS samengesteld door	: B.V. Centrale Aankoop FNZ	tel: (026) 3186711

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B.V. Centrale Aankoop FNZ; Graafsingel 22, 6921 RT Duiven, Postbus 93, 6920 AB Duiven
Telefoon 026 - 318 67 11, Fax algemeen 026 - 311 91 95. Handelsregister nr. 87792 te Arnhem.

ICS261
Nummer: A.3.
Versiedatum: 960507

Vacuum Zout

Natrium Chloride (NaCl) consumptiekwaliteit

FFP

Gedroogd, gezeefd consumptiezout, fijne fractie.

			specificatie	kenmerkende analyse	analyse methode
NaCl	(% basis droge stof	min.	99 . 9	99 . 92	Sma 775.01
H_2O	(%)	max.	0 . 15	0 . 07	Sma 280.15
SO_4	(%)	max.	0 . 08	0 . 07	Sma 292.02
Ca	(mg/kg)	max.	15	10	Sma 792.02
Cu	(mg/kg)	max.	0 . 10	0 . 01	Sma 792.02
Fe	(mg/kg)	max.	2 . 0	1 . 0	Sma 104.03
Mg	(mg/kg)	max.	1 . 0	0 . 5	Sma 792.02
Antikontermiddel(536)	(mg/kg)	min.	2	8	Sma 251.01
		max.	10		

		specificatie (%)	kenmerkende analyse (%)	
>1.0 mm		0	0	Sma 721.04
0.25-1.0 mm	max.	9	5	
0.125-0.25 mm	min.	77	83	
<0.125 mm	max.	14	12	

d50 0.18 mm

Door ontmenging tijdens opslag en transport zowel intern als extern kunnen separate monsters hiervan afwijken.

1200 - 1400 per kubieke meter

50 kg of 25 kg papier ventiel zakken
per silowagen min. 25 ton

zak 50 kg:	8715800907351	zak 25 kg:	8715800100134

Pallet voorzien van polyethyleen krimphoes

50 kg zakken:	25 kg zakken:	25 kg zakken
25 zakken per pallet	49 zakken per pallet	49 zakken per pallet
5 lagen per pallet	7 lagen per pallet	7 lagen per pallet
5 zakken per laag	7 zakken per laag	7 zakken per laag
netto: 1250 kg	netto: 1225 kg	netto: 1225 kg
bruto: ca 1293 kg	bruto: ca 1256 kg	bruto: ca 1272 kg
120 x 100 cm	120 x 100 cm	100 x 120 cm
Akzo retour pallet	Kunststof retour pallet	Akzo retour pallet
127 x 105 x 90 cm	120 x 100 x 96	120 x 100 x 96

Brussels nomenclature
2501.00.910
CAS registration
Nr. 7647-14-5

000349

Safety-information sheet (according to EG-standards 93/112/EEG)
Butter salt FFP

1. Identification

Product name	Butter salt FFP
Usual chemical name	Sodium chloride (NaCl)
Supplier	BV Centrale Aankoop FNZ
Phone Number for	National poison information center, emergency phone nr 030-2748888, (exclusive for treating physician for poisoning)

2. Composition and information about the components

Information about dangerous components

Chemical name	Sodium chloride
CASnr.	7647-14-5
% W/W	±100
EG-nr.	2315983
Symbol	none
R-sentences	none
Annex-1 nr	none

3. Danger identification

Not classified as a dangerous substance according to the EG-standards and the EG-standards for dangerous specimen

4. Emergency measures

Symptoms and effects	irritating to the eye
When swallowed	get s.o. to vomit
When inhaled	n.a.
Skin contact	rinse with water
Eye contact	rinse with a lot of water. See a doctor when irritated
Advice to doctor	none specific

5. Fire protective measures

Extinguishers	
Use	all media
Not to use	none
Unusual fire/explosive risks	in case of fire and/or explosion avoid inhalation
Dangerous thermal decomposition, and burning rests	Poisonous vapor (chlorine) develops in a burning environment

6. Measures during accidental release of the component

Personal measures	no specific recommendations
Environmental measures	collect as much as possible in a clean container, to drain or (better) reuse
Cleaning methods	sweep and put in container, rinse rest with a lot of water

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7. Handling and storage

Handling	no specific recommendations
Storage	store in a dry place (max. relative humidity=75%)
Fire-and explosive prevention	n.a.

8. Exposure control measures/personal protection

Professionally exposure limits	no limits
Personal protection	
Breathing system	n.a.
Skin and body	n.a.
Hands	n.a.
Eyes	protection glasses

9. Physical and chemical characteristics

Presentation	crystal
Color	white
Odor	none
Boiling point	1465°C
Melting point	801°C
pH	7,5 (18°C)
Water solubility	310g/l (18°C)
Solubility in other solvents	soluble in glycerol
Density	2170 kg/m^3 (20°C)
Flash point	n.a.
Flammability	not flammable
Self-ignition temp.	n.a..
Explosion margins	n.a.
Explosion qualities	none
Oxidizing qualities	n.a.
Bulk density	± 1250kg/m^3
Distribution coefficient	
n-octanol/water	log Pow = -3.0 (calculated)
relative steam density	n.a. (air = 1)
Viscosity	n.a.
Steam pressure	n.a.
Stability	stable when handled and stored according to section 7
Circumstances to avoid	heavy reaction with BrF_3
Substances to avoid	metals (except stainless steel)
Dangerous decomposition products	if involved in fire (Cl and Na_2O)

11. Toxicological information

Chemical name	sodium chloride
Acute toxicity	
Through the mouth LD50	rat: 3000mg/kg
Stimulation	
Eye contact	eye irritating

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12. Ecological information

No information available

13. Processing of waste instructions

Product	no specific recommendations
Contaminated packing	no specific recommendations

14. Transportation information

Un N°	none
Land-road/rail	
ARD/RID	
Class	not restricted
Number	n.a.
Dangerous indication nr	n.a.
TREM-card	n.a.
Proper shipping name	n.a.
Material indication nr	n.a.
Sea	
IMO/IMDG	
Class	not restricted
Code	not restricted
Packing group	n.a.
EMS	n.a.
MFAG	n.a.
Sea polluting material	no
Proper shipping name	n.a.
Air	
ICAAO/IATA/DGR class	not restricted
Packing group	n.a.
Proper shipping name	n.a.

15. Information required by law

EG rules	
Chemical description	sodium chloride (NaCl)
Classification according to	EG-rules
Dangerous symbol	none
Warning sentences	none
Water and danger class (WKG) (Germany)	O (evaluation water endangering materials committee)

16. Remaining information

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Warning:	Information in the above are gathered with the best possible consideration out of existing literature. BV Centrale Aankoop FNZ is not responsible for damage, in any way or size, that could result from a use of this information.

History

First issue date	October 12th 1993
Issue date	October 12th 1993
Version	1
SDS made up by	B.V. Centrale Aankoop FNZ Tel 026-3186711

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Vacuum salt

Sodium chloride (NaCl) consumption quality

Brand name **FFP**

Product description: Dried, sieved consumption salt, fine fraction

Composition

	specification	typical analysis	analysis method
NaCl(%dry)	min 99 . 9	99 . 92	Sma 775.01
H_2O (%)	max 0 . 15	0 . 07	Sma 280.15
SO_4 (%)	max 0 . 08	0 . 07	Sma 292.02
Ca (mg/kg)	max 15	10	Sma 792.02
Cu (mg/kg)	max 0 . 10	0 . 01	Sma 792.02
Fe (mg/kg)	max 2 . 0	1 . 0	Sma 104.03
Mg (mg/kg)	max 1 . 0	0 . 5	Sma 792.02
Anti clot 536 (mg/kg)	min 2 max 10	8	Sma 251.01

Particle size distribution

	specification (%)	typical analysis (%)	
>1.0 mm	0	0	Sma 721.04
0.25-1.0 mm	max 9	5	
0.125-0.25 mm	min 77	83	
<0.125 mm	max 14	12	
d50		0.18mm	

Due to storage and transportation, internal as well as external, separate samples can be less homogeneous.

Bulk density: 1200-1400 per m^3

Packaging: 25 or 50 kg paper valve bags
Per silo truck min. of 25 ton

EAN-code: bag 50kg 8715800907351
Bag 25kg 8715800100134

Packaging unit: Pallet provided with polyethylene shrink-wrap

	50kg bags 25 bags per pallet 5 layers per pallet 5 bags per layer	25kg bags 49 bags per pallet 7 layers per pallet 7 bags per layer	25kg bags 49 bags p pallet 7 layers p pallet 5 bags p layer
Weight	net:1250kg Gross:ca1293kg	net:1225kg gross:ca1256kg	net:1225kg gross:ca1272kg
Pallet size	120x100cm Akzo return pallet 127x105x90cm	120x100cm plastic return pallet 120x100x96cm	100x120cm Akzo return pall 120x100x96cm

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Product code : 38120
Product name : Phosphoric acid
Date : 12-15-1998
Edition nr. : 01
Status : Final

Description : Clear color- and odorless fluid.
Specific for process bio- active proteins.

Analysis code	Reference	Value
0457	Phosphoric acid	min. 85 %
0552	Phosphoric pentoxide. (P205)	61 +/- 1.5%
0979	pH general	2
	volatile acids	max.10ppm
1543	potassium permanganate	max. 10mg/l
1161	sulfate	max. 50mg/g
0447	fluoride	max. 1mg/kg
0546	nitrate	max. 5mg/kg
0432	chloride	max. 2ppm
0494	copper	max. 1mg/kg
0575	zinc	max. 2mg/kg
0419	arsenic	max. 0.5mg/kg
0425	calcium	max. 50mg/kg
0422	cadmium	max. 1000ppb
0440	cobalt	max. 1mg/kg
0485	potassium	max. 5mg/kg
0510	magnesium	max. 10mg/kg
0570	iron	max. 10mg/kg
0517	manganese	max. 1mg/kg
0530	sodium	max. 500mg/kg
0548	nickel	max. 1mg/kg
0505	lead	max. 1mg/kg
	antimoon	max. 5mg/kg

Remarks:

1 The product of this specification apply to the requirements of the Nederlandse levensmiddelenwetgeving. (Warenwet. produktschapsverordeningen etc. naar gelang het geval)

000355

22-10-99

Caldic Nederland BV
Blaak 22
3011 TA ROTTERDAM
Tel: 010-4136420
Fax: 010-4047458
E-Mail: d.kobes@caldic.nl

DMV International
NCB- Laan 80
T.a.v: dhr C.J.M. Habraken

Geachte heer Habraken,

Herewith we Caldic Nederland BV declare that our Phosphoric Acid type 75% fulfills all applicable EC requirements:
Manufactured to satisfy the purity requirements of:
UK- Miscellaneous Additives in Food Regulations (1980) for E 338 (78/664/EEC).
USA- Food Chemicals Codex (1981)
Food Agricultural Organisation/ World Health Organisation (1978).

Wij hopen u hiermee voldoende te hebben geïnformeerd en verblijven wij,

Met vriendelijke groet,

Caldic Nederland BV

Dennis Kobessen

Bijlage: 2 stuks(spec)

CALDIC THE RIGHT CHEMISTRY

000356

CALDIC

caldic belgium nv
terlochtweg 1
B-2620 hemiksem

tel. (03) 870 48 11
telex 31681
fax (03) 870 48 29

v.a.t. 403.671.933

PRODUCT SPECIFICATION

PHOSPHORIC ACID 75% FOOD GRADE

Assay as P2O5	53,8 - 54,8% weight
Density at 15,5 °C	about 1,585
As	max. 2 ppm
Pb	max. 5 ppm
SO4	max. 250 ppm
F	max. 10 ppm
Cl	max. 30 ppm
NO3	max. 5 ppm
Zn	max. 25 ppm
Cu	max. 25 ppm
Heavy metals	max. 10 ppm
Volatile acids as acetic acid	max. 10 ppm
Colour	Clear and colourless

08.01.96

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ALBRITE PHOSPHORIC ACIDS AND POLYPHOSPHORIC ACIDS

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ALBRIGHT & WILSON

FOOD GRADE

This grade of orthophosphoric acid is suitable for applications in the food and drinks industries, and applications where a high degree of purity is demanded. Food grade acid can be manufactured to higher qualities than indicated below in order to meet customers' specific requirements. We shall be pleased to discuss the provision of special grades of food quality acid on request.

A specification and typical analysis are shown below:-

Manufactured to satisfy the purity requirements of **UK**-Miscellaneous food additive Regulations (1995) for **E338** (78/664/EEC). Food Agricultural Organisation/World Health Organisation (1978): CEN-Chemicals used for treatment of Water intended for Human Consumption

Appearance	Clear, liquid		
Available concentrations			
Grade	850 F	812 F	750 F
H_3PO_4 % w/w	85	81·2	75
P_2O_5 % w/w	61·6	58·8	54·3

	Typical	**Specification**
P_2O_5 % w/w	as detailed above	±0·5 on all concentrations
Colour, Hazen units	20	
As ppm	0·2	2 max
Pb ppm	0·5	5 max
SO_4 ppm	100	250 max
F ppm	4	10 max
Cl ppm	< 10	30 max
NO_3 ppm	< 5	5 max
Zn ppm	< 2	25 max
Cu + Zn ppm	< 3	50 max
Heavy Metals ppm	< 10	10 max
Fe ppm	< 4	
Volatile acids as acetic acid ppm	< 10	10 max

PURIFIED GRADE

This product is a high quality solvent extracted acid suitable for general industrial applications. A specification and typical analysis are shown below -

Appearance	Clear, pale to straw coloured liquid		
Available concentrations			
Grade	850 P	812 P	750 P
H_3PO_4 % w/w	85	81·2	75
P_2O_5 % w/w	61·6	58·8	54·3

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	Typical	**Specification**
P_2O_5 % w/w	as detailed above	±0·5 on all concentrations
As ppm	2	3 max
Pb ppm	1	5 max
SO_4 ppm	400	500 max
F ppm	10	20 max
Heavy Metals ppm	10	20 max
Fe ppm	20	

Product code : 18111
Product name : Demineralised water
Date : 07-07-1995
Edition nr : 02
Status : Final

Analysis code	Make	Value
0039	conductivity	max. 50 µS
0097	temperature	max. 10°C
0475	total solids	max. 10ppm
0979	pH general	5.0-7.0
1516	iron	max. 0.01mg/l
1513	nitrate	max. 1mg/l
1512	nitrite	max. 0.025mg/l
1519	copper	max. 3ug/l
1521	zinc	max. 5ug/l
0418	ammonia	max. 0.05mg/kg
1506	chloride (Mohr)	max. 0.1mg/l
1507	sulfate (water)	max. 1mg/l
1515	silicate	max. 0.05 mg/l
1508	calcium (water)	max. 0.05 mg/l
1545	sodium (water)	max. 0.2mg/l
1509	magnesium (water)	max. 0.01mg/l
1563	plate count 22°C 72 hours	max. 100cfu/ml
1562	plate count 37°C 48 hours	max. 10cfu/ml
0764	e.coli in 300ml	negative
0744	coliforms in 300ml	negative

Remarks:

- The product of this specification apply to the requirements of the Nederlandse levensmiddelenwetgeving. (Warenwet, produktschapsverordeningen etc. naar gelang het geval)

000360

Product code : 38075
Product name : Di-sodiumphosphate 12AQ N5206= $Na_2HPO_4.12H_2O$.
Date : 08-22-1996
Edition nr : 02
Status : Final

Description : clear. crystal powder. EG acceptance nr. E 339
Manufacture NR. N52-06

Analysis code	Reference	Value
0543	sodium phosphate sec	98.0-101 %
0118	moisture acc. to KF	57.0-61.0%
0552	phosphoric pentoxide.	19.5-20.5%
0540	sodium oxide.	16.5-17.5%
0979	pH general	9.1-9.4
0120	scorched particles	disc 1
0046	insoluble materials	max. 0.1%
0041	odor	max. "+" odorless
0051	color	max. "+" colorless
0151	fineness <180um	max. 5%
0148	fineness<1000um	min. 70 %
0878	fines<2000um	min. 99 %
0432	chloride	max. 200 ppm
0447	fluoride	max. 3mg/kg
1162	sulfate	max. 0.05%
0570	iron	max. 20 mg/kg
0581	heavy metals	max. 10 mg/kg
0419	arsenic	max. 1mg/kg
0505	lead	max. 1mg/kg
0575	zinc	max. 25 mg/kg
0496	copper and zinc	max. 50 mg/kg

Remarks:

1 Storage in a dry place. in closed bags.
2 The product of this specification complies with the requirements of the Nederlandse levensmiddelenwetgeving. (Warenwet. produktschapsverordeningen etc. naar gelang het geval).

000361

DINATRIUMPHOSPHAT 2-HYDRAT
krist.
Lebensmittelqualität

Produkt-Nr.:	N 12-20
Chemische Bezeichnung:	Dinatriummonohydrogen-monophosphat-Dihydrat
Chemische Formel:	$Na_2HPO_4 \cdot 2H_2O$
Molekulargewicht:	178
CAS-Nr.:	7558-79-4 (für Anhydrid)
EINECS-Nr.:	2314487
EG-Nr.:	E 339
Beschaffenheit:	farblose Kristalle

Typische Analyse:

Na_2O (%):	34,8
P_2O_5 (%):	40
Trockenverlust (%): (1h 60°C, 4h 105°C)	20
pH-Wert (1%):	9,2
Arsen (ppm):	< 1
Blei (ppm):	< 1
Eisen (ppm):	1
Chlorid (ppm):	10
Fluorid (ppm):	1
Sulfat (ppm):	10
H_2O-unlöslicher Anteil (%):	< 0,1
Schüttgewicht (g/l):	1000
Siebung:	
auf 0,850 mm (%):	6
auf 0,425 mm (%):	60
durch 0,150 mm (%):	15
Löslichkeit (g/100 g H_2O):	
10°C	6
25°C	15
50°C	102

Die wässrige Lösung von Dinatriumphosphat reagiert alkalisch. Beim Erhitzen von Dinatriumphosphat bildet sich Tetranatriumpyrophosphat. Bei der Umsetzung mit Schwermetallsalzen entstehen schwerlösliche Phosphate. Dinatriumphosphat hat ausgezeichnete Puffereigenschaften.

Vertegenwoordigd door:

C.N. SCHMIDT B.V.
POSTBUS 9696
1006 GD AMSTERDAM

TEL.: 020 - 619 64 44
FAX: 020 - 619 57 26
E-MAIL: cns@cnschmidt.nl

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Chemische Fabrik BUDENHEIM Rudolf A. Oetker

Chemische Fabrik Budenheim Rudolf A Oetker
D-55257 Budenheim Rheinstraße 27
D-55253 Budenheim Postfach 1147-1149
Telefon ++49 +61 39 89 0 Telefax ++49 +61 39 89 264

DINATRIUMPHOSPHAT 2-HYDRAT
krist.
Lebensmittelqualität

Hauptanwendungsgebiete:

- Biotechnologie
- Email
- Farben
- Fermentation
- Futtermittel
- Metallbehandlung
- Nahrungsmittel
- Pharmazie
- Reinigungsmittel
- Textil
- Wasseraufbereitung

Sonderspezifikationen für pharmazeutische Zwecke können auf Wunsch geliefert werden.

Verpackung

- Säcke 25 kg netto

Lagerbedingungen

- kühl und trocken lagern

Sonderspezifikationen für pharmazeutische Zwecke können auf Wunsch geliefert werden.

Mit diesen Angaben über unser Produkt und dessen Verwendungsmöglichkeiten wollen wir Sie nach bestem Wissen beraten. Die Angaben werden hiermit jedoch nicht verbindlich zugesichert, sondern müssen für die jeweilige konkrete Anwendung überprüft werden.

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5/95

Chemische Fabrik Budenheim Rudolf A Oetker
D-55257 Budenheim Rheinstraße 27
D-55253 Budenheim Postfach 1147-1149
Telefon ++49 +61 39 89 0 Telefax ++49 +61 39 89 264

Chemische Fabrik
BUDENHEIM
Rudolf A. Oetker

Chemische Fabrik
BUDENHEIM
Rudolf A. Oetker

cfb Postfach 1147-1149 D-55253 Budenheim

Chemische Fabrik Budenheim
Rudolf A Oetker
Rheinstraße 27
D-55257 Budenheim
Telefon ++49 +61 39 89 0
Telefax ++49 +61 39 89 264

to whom it may concern

Ihr Schreiben vom Zeichen Your Reference	Unser Zeichen Our Reference	Durchwahl Direct Line	Datum Date
	V1/Fb	06139/89-437	05.07.99

ORIGIN - HEALTH CERTIFICATE

Phosphates produced by Chemische Fabrik Budenheim, Rudolf A. Oetker

We, Chemische Fabrik Budenheim, Rudolf A. Oetker, hereby certify that all our phosphates are produced according to the current rules of the Federal Republic of Germany and that they can be supplied for human consumption.

We furthermore declare that our phosphates do not content any harmful substances and that our installation plants are in perfect hygienic condition which corresponds to the demands of our Health Authorities.

Chemische Fabrik Budenheim
Rudolf A. Oetker

i.A. Ferber i.A. Jauch

000364

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Bielefeld Bankhaus Hermann Lampe KG
BLZ 480 201 51
Konto-Nummer 90 190

Landeszentralbank Mainz
BLZ 550 000 00
Konto-Nummer 550 073 51

Mainzer Volksbank Mainz
BLZ 551 900 00
Konto-Nummer 162 744 015

Commerzbank AG, Mainz
BLZ 550 400 22
Konto-Nummer 210 248 100

Deutsche Bank AG, Mainz
BLZ 550 700 40
Konto-Nummer 106 757

Dresdner Bank AG, Mainz
BLZ 550 800 65
Konto-Nummer 252 300 000

USt D-Nr DE 149 024 20

Productbenaming: Dinatriumfosfaat 2-hydraat

1. PRODUCT-/TOEBEREIDINGS- EN FIRMABENAMING

1.1 **Gegevens over het product**
Productbenaming: Dinatriumfosfaat 2-hydraat
Productnr.:

1.2 **Gegevens over de fabrikant/leverancier**
1.2.1 Chemische Fabrik Budenheim, Rudolf A. Oetker
Rheinstraße 27, D-55257 Budenheim
Tel.: 06139-89-0

1.2.2 **Infotelefoonnummer**
Verantwoordelijke voor gevaarlijke producten, tel. 06139-89261

1.2.3 **Noodtelefoonnummer:** 06139-89414

2. SAMENSTELLING/GEGEVENS VAN BESTANDDELEN

2.1 **Chemische kenmerken**
Dinatriumfosfaat 2-hydraat
CAS-nr.: 7558-79-4 EINECS-nummer: 2314487
E-nr.:

2.2 **Gevaarlijke bestanddelen**

2.3. **Extra richtlijnen**

3. MOGELIJKE GEVAREN

3.1 **Gevarensymbolen**
Geen gevaarlijke stoffen volgens de verordening voor gevaarlijke stoffen/EG-richtlijnen

3.2 **Maatregelen ter bescherming van het milieu**
Niet in de riolering en in de wateren terecht laten komen

4. EERSTE-HULP-MAATREGELEN

4.1 **Algemene richtlijnen**

4.2 **Na het inademen**

4.3 **Na huidcontact**
Aangetaste huid met veel water wassen.

4.4 **Na oogcontact**
Ogen onmiddelijk meerdere minuten onder stromend water met geopende oogspleet spoelen.

4.5 **Na het inslikken**

4.6 **Richtlijnen voor de arts**

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5. BRANDBESTRIJDINGSMAATREGELEN

5.1 Geschikte blusmiddelen
Alle blusmiddelen geschikt

5.2 Blusmiddelen die om veiligheidsredenen niet geschikt zijn
Niet bruikbaar

5.3 Bijzonder gevaar door het product of de toebereiding

5.4 Persoonlijke veiligheidssuitrusting
Nauw aansluitende veiligheidsbril dragen
Geschikte veiligheidshandschoenen van rubber of kunststof dragen

6. MAATREGELEN BIJ HET PER ONGELUK VRIJKOMEN VAN PRODUCTEN

6.1 Persoonlijke maatregelen

6.2 Maatregelen ter bescherming van het milieu
Niet in de riolering en in de wateren terecht laten komen

6.3 Reinigung en opname
Mechanisch opnemen, resten met veel water spoelen

7. BEHANDELING EN OPSLAG

7.1 Behandeling

7.1.1 Richtlijnen ter beveiliging tegen brand en explosie
Geen bijzondere maatregelen noodzakelijk

7.2 Opslag
Koel, groog en stevig gesloten bewaren

8. OPSTELLINGBEPERKING EN PERSOONLIJKE VEILIGHEIDSSUITRUSTING

8.1 Bijkomende richtlijnen voor de opstelling van techn. installaties

8.2 Bestanddelen met te controleren grenswaarden

8.3 Persoonlijke veiligheidsuitrusting

8.3.1 Werkhygiene
De bij de omgang met chemicalien gebruikelijke gedragsregels in acht nemen.

8.3.2 Adembescherming

8.3.3 Handbescherming
Handschoenen van kunststof of rubber

8.3.4 Oogbescherming
Veiligheidsbril

8.3.5 Lichaamsbescherming

000366

Productbenaming: Dinatriumfosfaat 2-hydraat

9. FYSISCHE EN CHEMISCHE EIGENSCHAPPEN

9.1 Uiterlijke kenmerken

9.1.1 Kleur: kleurloos

9.1.2 Vorm:

9.1.3 Reuk: reukloos

9.2 Veiligheidsrelevante gegevens

Smeltpunt	ca 95 °C
Warmte ontbinding	> 250 °C
Vlampunt	niet van toepassing
Ontstekingstemperatuur	niet van toepassing
Ontploffingsgrenzen:	
Ontploffingsgrens (ondere)	niet van toepassing
Ontploffingsgrens (oppere)	niet van toepassing
Dichtheid	2,066 g/cm3 bij 20 °C
Oplosbaarheid in water	60 g/l bij 10 °C
pH-waarde	9,2 bij 20 °C bij 10 g/l H2O

9.3 Andere gegevens

Geen gegevens

10. STABILITEIT EN REACTIVITEIT

10.1 Te vermijden omstandigheden

10.2 Te vermijden stoffen

10.3 Gevaarlijke ontledingsstoffen

Geen

10.4 Gevaarlijke reacties

Bij een deskundig gebruik en opslag geen.

11. GEGEVENS OVER TOXICOLOGIE

11.1 Indelingsrelevante LD/LC 50-waarden

11.2 Gevaar voor de gezondheid/werking

000367

Productbenaming: Dinatriumfosfaat 2-hydraat

12. GEGEVENS OVER ECOLOGIE

12.1 **Persistentie en afbreckbaarheid**
Bij een deskundig gebruik zijn er volgens onze ervaringen geen storingen bij de zuivering van het afvalwater te verwachten. Het product mag echter niet in grote hoeveelheden in het afvalwater terechtkomen.

12.2 **Mobiliteit**
Het product ist een wateroplosbare vaste stof

12.3 **Aquatische toxiciteit**

12.4 **Bijkomende richtlijnen**

12.5 **Ecologie algemeen**
Zwak watergevaarlijke stof

13. RICHTLIJNEN VOOR DE AFVOER

13.1 **Product**
Dinatriumfosfaat 2-hydraat
Afvoer bij ongebruikt materiaal
Recycling
Afvoer bij gebruikt materiaal
Recycling

13.2 **Afvoer van vervuilde verpakkingen**
Afvoer als REPA-zak

13.3 **Afvalcodenummer**
060307 Fosfaten en verwante zouten

14. GEGEVENS VOOR HET TRANSPORT

14.1 **ADR/GGVS:**
14.1.1 ADR/RID-klasse: n.a 14.1.2 Cijfer: n.a.
14.1.3 Kemmler-nr.: 00 14.1.4 UN-nr.: 0000

14.2 **ADN(R):**
14.2.1 ADNR-klasse: n.a. 14.2.2 Cijfer: n.a.

14.3 **IMDG code/GGVSee:**
14.3.1 IMMDG/GGVSee-klasse: n.a 14.3.2 Verpackingsgroep:
14.3.3 EMS: 14.3.4 MFAG: n.a.
14.3.5 Marine Pollutant: 14.3.6 Gevaarl. stof. uitzond.VO: n.a.
14.3.7 Proper shipping name: Kein Gefahrgut, fest/no dangerous good

14.4 **IATA DGR:**
14.4.1 ICAO/IATA-klasse: n.a
14.4.2 Passenger Aircraft: J 14.4.3 Passenger Verp. Instr.:
14.4.4 Cargo Aircraft: 14.4.5 Cargo Verp. Instr.:
14.4.6 Primair label: n.a. 14.4.7 Secundair label: n.a.
14.4.8 Andere uitzonderingen:
14.4.9 Proper shipping name:

000368

Productbenaming: Dinatriumfosfaat 2-hydraat

15. VOORSCHRIFTEN

15.1 Kenmerking volgens EG-richtlijnen

15.1.1 Kenletter en gevaarenbenaming van het product
E-nr.:
Geen gevaarlijke stoffen volgens de verordening voor gevaarlijke stoffen/EG-richtlijnen

15.1.2 Gevaarbepalende componenten voor de etikettering

15.1.3 R-reeksen

15.1.4 S-reeksen

15.2 Nationale voorschriften

15.2.1 List van de gevaarlijke stoffen volgens §4 GefStffV:
Nee

15.2.2 Bedrijfsbeperkingen:
Geen

15.2.3 In geval van storing: n.a.

15.2.4 VbF-klasse: n.a.

15.2.5 TA-lucht:

15.2.6 Watergevarenklasse: 1

15.2.7 Verdere voorschriften, beperkings- en verbodsverordeningen:
Afvalcodenummer
060307 Fosfaten en verwante zouten

16. VERDERE GEGEVENS

000369

DI SODIUM PHOSPHATE 2-HYDRATE, cryst,
Food grade quality

Product nr:	N12-20
Chemical name:	di sodium mono hydrogen mono phosphate di hydrate
Chemical formula:	$Na_2HPO_4 - 2H_2O$
Molecular weight	178
CAS-nr:	7558-79-4 (for anhydrate)
EINECS-nr:	2314487
Eg-NR:	E339
Quality:	colorless crystal

	Typical analysis
Na_2O (%)	34,8
P_2O_5 (%)	40
Dry loss (%) (1h 60°C, 4h 105°C)	20
pH-value (1%)	9,2
Arsenic	<1
Lead (ppm)	<1
Iron (ppm)	1
Chloride (ppm)	10
Fluoride (ppm)	1
Sulfate (ppm)	10
H_20 insoluble part (%)	<0,1
Bulk density (g/l)	1000
Particle size:	
On 0,850 mm (%):	6
On 0,425 mm (%)	60
Through 0,150 mm (%)	15
Solubility (g/100g H_2O):	
10°C	6
25°C	15
50°C	102

A watery solution of di sodium phosphate reacts alkaline. When di sodium phosphate is heated, there's tetra sodium pyro phosphate build up. When reacting with heavy metal salts slightly insoluble phosphates develop. Di sodium phosphate has outstanding buffer qualities.

Main application area:

-Biotechnology
-Enamel
-Colors
-Fermentation
-Feed
-Metal treatment
-Food
-Pharmacy

000370

-Cleaning agent
-Fabric, textile
-Water preparation

Special specifications for pharmaceutical applications are delivered on request.

Packaging
-bags of 25kg net

Storage conditions
-store cool and dry

Special specifications for pharmaceutical applications are delivered on request.

With these declarations about our product and it's applications, we would like to advise you to our best effort. We can offer no guarantees about applications, the use of the product has to be tailored to each application.

000371

Product name: Di sodium phosphate 2 hydrate

1 Product/manufacturing- and firm name

1.1 Facts about the product

Product name: di sodium phosphate 2-hydrate
Product number:

1.2 Facts about the manufacturer/supplier

1.2.1 Chemische Fabrik Budenheim, Rudolf A. Oetker, Rheinstrasse 27, D-55257 Budenheim, Tel 06139-89-0

1.2.2 Information phone nr.

The person responsible for hazardous products: tel. 06139-89261

1.2.3 Emergency phone nr.

06139-89414.

2 Composition/facts of elements

2.1 Chemical features

Di sodium phosphate 2-hydrate
Cas-nr.: 7558-79-4 EINECS-nr: 2314487
E-nr:

2.2 Hazardous components

2.3 Extra directions

3 Possible hazards

3.1 Hazard symbols

No hazardous elements according to EG standards

3.2 Environmental protection measures

Don't let it get into the sewer system or waters

4 Emergency measures

4.1 General directions

4.2 After inhaling

4.3 After skin contact

Rinse with a lot of water if skin is affected

4.4 After eye contact

Immediately rinse eyes (opened) with flowing water

4.5 After swallowing

4.6 Directions for physician

000372

5 Fire control measures

5.1 Appropriate extinguisher
Everything appropriate

5.2 Extinguishers not appropriate because of safety
Unusable

5.3 Particular hazard because of product or manufacturing

5.4 Safety equipment
Wear good fitting safety glasses, and rubber or plastic safety gloves

6 Measures if product is released by accident

6.1 Personal measures

6.2 Environmental protection measures
Don't let it get into the sewer or surface waters

6.3 Cleaning and absorption
Mechanical absorption; rinse the remains with a lot of water

7 Handling and storage

7.1 Handling

7.1.1 Fire and explosion safety guidelines
No special arrangements necessary

7.2 Storage
Storage cool, dry and closed

8 Placement limitation and personal safety equipment

8.1 Additional directions for placement of technical installations

8.2 Components with values to be controlled

8.3 Personal safety equipment

8.3.1 Work hygiene
Consider the usual rules when working with chemicals

8.3.2 Breath protection

8.3.3 Hand protection

8.3.4 Eye protection
Safety glasses

8.3.5 Body protection

9 Physical and chemical characteristics

9.1 External features

9.1.1 Color: colorless

9.1.2 Form:

9.1.3 Odor: odorless

9.2 Relevant safety data

Melting point	ca 95 °C
Warmth disintegration	>250 °C
Flash point	not applicable
Ignition temp.	not applicable
Explosion limits	
Explosion limit (lower)	not applicable
Explosion limit (upper)	not applicable
Density	2,066 g/cm3 at 20°C
Solubility in water	60 g/l at 10°C
pH value	9,2 at 20°C at 10g/l H_2O

9.3 Other data

No data

10. Stability and reactivity

10.1 Circumstances to avoid

10.2 Element to avoid

10.3 Dangerous segregation elements

None

10.4 Dangerous reactions

None when used and stored expertly

11. Toxicological data

11.1 Lay-out relevant LD/LC values

11.2 Health danger

12. Ecological data

12.1 Persistence and decomposability

No disturbance while purifying the wastewater is to be expected when used expertly. However, the product should not get into wastewater in large amounts.

12.2 Mobility

It's a water-soluble solid substance

12.3 Aquatic toxicity

000374

12.4 Additional directions

12.5 General ecology

Weak water hazardous substance

13 Disposal directions

13.1 Product

Di sodium phosphate 2-hydrate
Unused material drainage
Recycling
Used material drainage
Recycling

13.2 Polluted packing material disposal

Drain as REPA-bag

13.3 Waste code number

060307 phosphates and related salts

14 Transportation data

14.1 ADR/GGVS:

14.1.1 ADR/RID-class: n.a

14.1.2 Number: n.a

14.1.3 Kemmler number: 00

14.1.4 Un-nr.: 0000

14.2 ADN (R):

14.2.1 ADNR-class: n.a

14.2.2 Number: n.a

14.3 IMDG code/GGVSee:

14.3.1 IMMDG/GGVSee-class: n.a

14.3.2 Packing group:

14.3.3 EMS:

14.3.4 MFAG: n.a

14.3.5 Marine pollutant:

14.3.6 Hazardous elements except VO:n.a

14.3.7 Proper shipping name: Kein Gefahrgut, fest/no dangerous good

14.4 IATA DGR

000375

14.4.1 ICAO/IATA-class:n.a

14.4.2 Passenger Aircraft: J

14.4.3 Passenger packing instr:

14.4.4 Cargo aircraft:

14.4.5 Cargo packing instr:

14.4.6 Main label: n.a

14.4.7 Secondary label: n.a

14.4.8 Other exceptions:

14.4.9 Proper shipping name:

15 Regulations

15.1 Characterization according to EG-standards

15.1.1 ID letter and danger identification of the product
E-nr:
No hazardous elements according to EG standards

15.1.2 Danger determining components for labeling

15.1.3 R-sequence

15.1.4 S-sequence

15.2 National regulations

15.2.1 List of dangerous materials according $4 GefStffV: no

15.2.2 Plant limitation: none

15.2.3 In case of disturbance: n.a

15.2.4 VbF-class: n.a

15.2.5 TA-air:

15.2.6 Water dangerous class: 1

15.2.7 Further regulations, limitation and prohibition regulations:
Waste code number
060307 Phosphates and related salts

16 Further data

000376

Sterilox Ceramic Membrane

000377

ILLINOIS WATER TREATMENT COMPANY

INTRODUCTION

The Sterilox Ceramic membrane filter is designed to be reused and to maintain integrity after hundreds of chemical cleanings, acid depyrogenations, and autoclave or steam sterilization cycles.

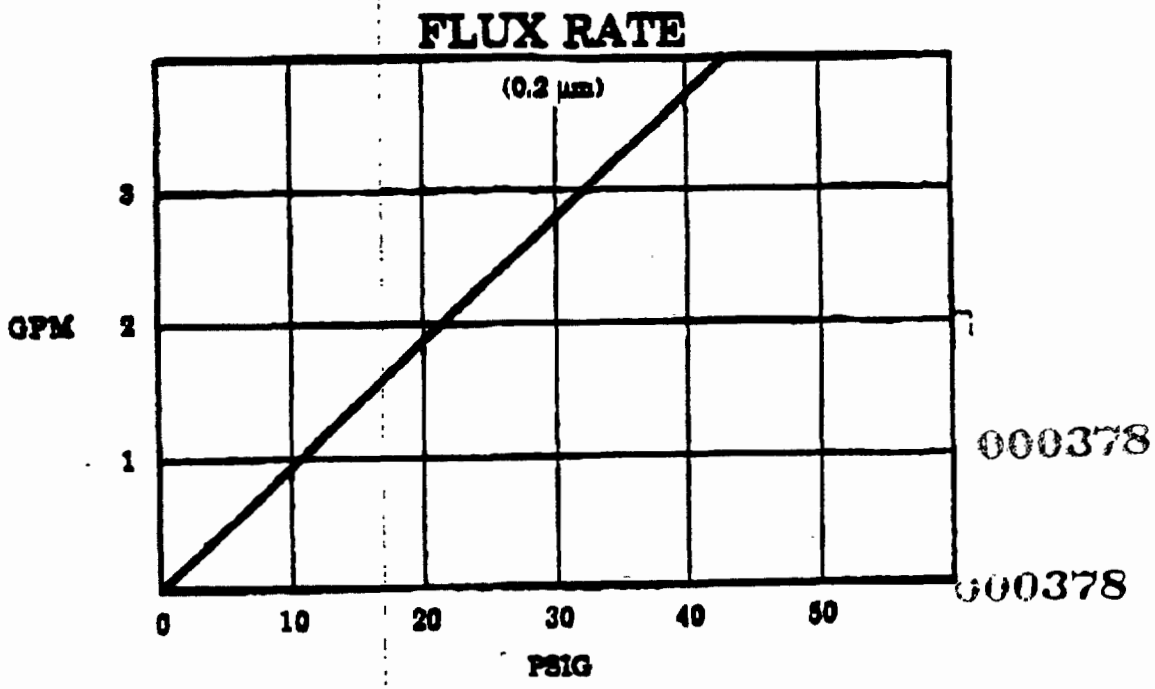
Each Sterilox membrane filter is shipped with a full diffusive flow integrity test curve. Additionally, each Sterilox element can also be shipped with a certificate of bacteria retention for that particular element.

Product Description:

Filter:	Pure Alpha Alumina multi-channel element
Gaskets:	Silicone; Buna N, Viton A or EPDM
Housing:	Pharmaceutical finish 316L SS housing
Filtration Area:	2.1 ft^2 per element

Operating Characteristics:

Maximum Temperature:	Limited by gasket material (400°F)
Maximum Pressure:	Limited by housing rating
Maximum Back Pressure:	Limited by housing rating
Flow Rate:	1.3 gpm @ 14.7 psig differential



000378
000378

ILLINOIS WATER TREATMENT COMPANY

BACTERIAL RETENTION TESTS OF 0.2 um STERILOX ELEMENTS

IWT offers Sterilox elements that are certified for bacterial retention using the established testing guidelines described in Appendix D. Twenty Sterilox elements that have passed the bacterial challenge and their respective diffusive flow integrity test curves are included in Appendix C and B, respectively.

Methodology:

Before the challenge test, each of the twenty elements was steam sterilized by autoclave for 45 minutes. After wetting, each element was integrity tested at 80 psid. P. Diminuta challenge suspensions were prepared as described in Appendix D to a concentration of at least 10^{10} organisms per element. The suspension was fed from a pressure tank through the element at a differential pressure greater than 30 psid.

Sterilox Retention Data:

All of the Sterilox elements retained the challenge organism. Certified reports for each of these elements are included in Appendix C. Diffusion test data was reported in drops of downstream liquid per minute and converted to cc/min by dividing drops per minute by 20.

Sterilox Element #	Challenge Concentration	Diffusion Test @ 80 psig	Log Reduction Value
E11402	2.1 x 10*10	.75 cc/min	10.3
E11403	9.9 X 10*10	.6 cc/min	11.0
E11406	2.7 X 10*10	.5 cc/min	10.4
E11412	2.8 X 10*10	.75 cc/min	10.5
E11416	2.0 X 10*10	.5 cc/min	10.3
E11417	2.0 X 10*10	.45 cc/min	10.3
E11418	2.1 X 10*10	.7 cc/min	10.3
E11404	2.6 X 10*10	.95 cc/min	10.4
E11400	3.6 X 10*10	2.2 cc/min	10.6
E11413	1.6 X 10*10	2.25 cc/min	10.2
E11408	8.4 X 10*10	.5 cc/min	10.9
E11411	1.0 X 10*11	.85 cc/min	11.0
E11401	2.5 X 10*10	.3 cc/min	10.4
E11410	3.9 X 10*10	.2 cc/min	10.6
E11405	4.5 X 10*10	1.85 cc/min	10.7
E11409	7.2 X 10*10	2.0 cc/min	10.9
E11407	2.3 X 10*10	.8 cc/min	11.4
E11415	1.0 X 10*11	1.05 cc/min	11.0
E11414	5.4 X 10*10	.8 cc/min	10.7
E11399	9.0 X 10*10	.95 cc/min	11.0

000379

BACTERIAL RETENTION TEST OF 0.2 µm STERILOX ELEMENT (continued)

RETENTION EFFICIENCY OF 0.1 µm ORGANISMS BY A 0.2 µm STERILOX ELEMENT:

Alcoa Separations had a typical 0.2 µm Sterilox element tested for retention of a 0.1 µm diameter Mycoplasma capricolum at a challenge level of 10^7 organisms per cm^2 of filtration area.

Methodology:

A 0.2 µm Sterilox element was tested for 0.1 µm organism retention utilizing the test procedure documented in Appendix F. The test equipment is identical to that used for HIMA guideline challenges utilizing Pseudomonas diminuta.

Sterilox Retention Data:

The tested Sterilox element demonstrated significant retention of the challenge organism. Certified reports for this element are included in Appendix F. Diffusion test data was previously reported in Appendix C.

Sterilox Element#	Challenge Concentration	Diffusion Test @ 80 psig	Log Reduction Value
E11416	$1.2 \times 10^{[illegible]}$	.5 cc/min	8.4

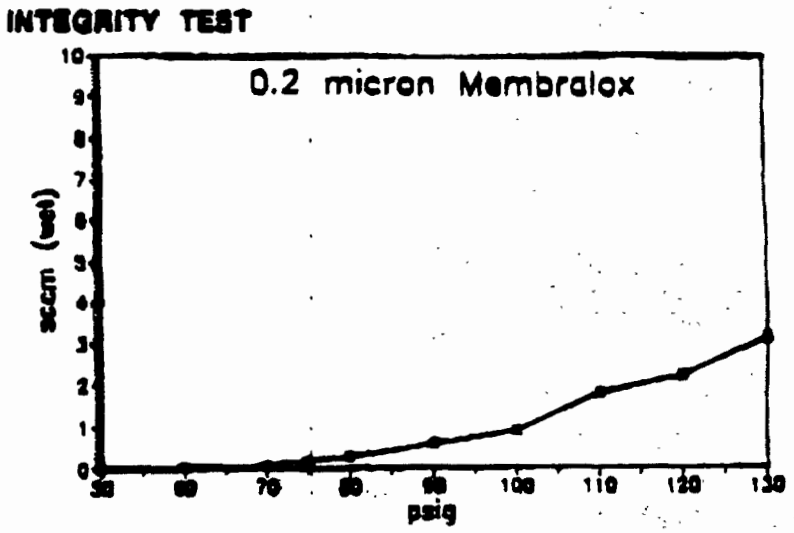
000380

STERILOX DIFFUSIVE FLOW INTEGRITY TESTS

Each Sterilox diffusive flow versus pressure curve (Figure 1; Appendix B) is characterized utilizing a Mass Flow Transducer Based Filter Integrity Test Instrument.

Many of the curves demonstrated negative diffusive flow numbers at pressures less than 50 psig. If we rerun the diffusive flow curve, wait ten minutes at each step, the element will demonstrate zero diffusion at pressures less than 50 psig but high diffusive flows at pressures greater than 50 psig as the membrane surface begins to dry. IWT has chosen to accept the negative diffusive flow numbers at pressures less than 50 psig in order to maintain accuracy at pressures greater than 50 psig.

The reported bubble points are calculated by examining the first deflection of diffusive flow for the element even though none of the elements is demonstrating bulk gas flow in the reported region. Conventional bubble point tests would suggest bulk flow at gas pressures approximately equal to 150 psig.



000381

PREWETTING TECHNIQUES FOR THE STERILOX MEMBRANE

There are two recommended methods of wetting a Sterilox element. Dynamic and Static Wetting.

Static Wetting:

Fill the upstream side of the element with water and allow the membrane to soak for 2 hours before pressurizing the system. If the membrane is allowed to soak for less than two hours, the element will demonstrate higher diffusive flow rates (Figure 4).

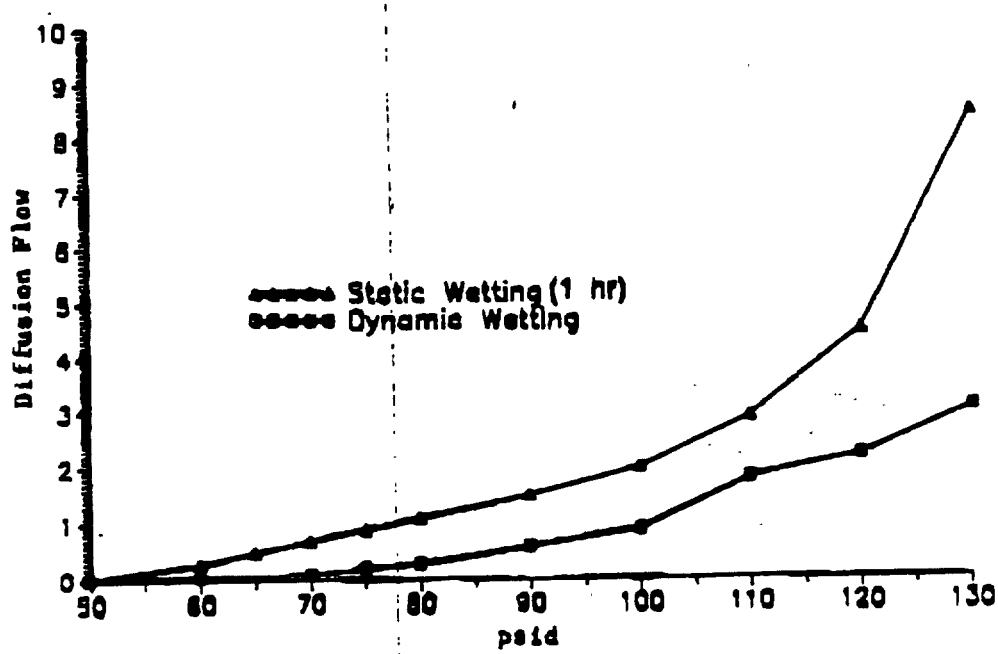
Dynamic Wetting:

Slowly fill the upstream side of the element with the permeate valves closed. Increase the pressure of the upstream side and hold until the permeate pressure approximately equals the feed pressure (less than 30 minutes). Open the permeate valve and start the filtration run.

Figure 4

DIFFUSION FLOW TEST

0.2 micron Sterilox



000382

TOXICITY TEST OF THE STERILOX ELEMENT

The Sterilox element was evaluated for pharmaceutical solution contact suitability using the USP Class VI Toxicology Test. Representative samples of the element were submitted to Sterilization Technical Services, Inc. of Rush, NY. The final test results demonstrate that all samples passed the USP Class VI Toxicology Test (Appendix A).

PYROGENICITY OF THE STERILOX MEMBRANE ELEMENT

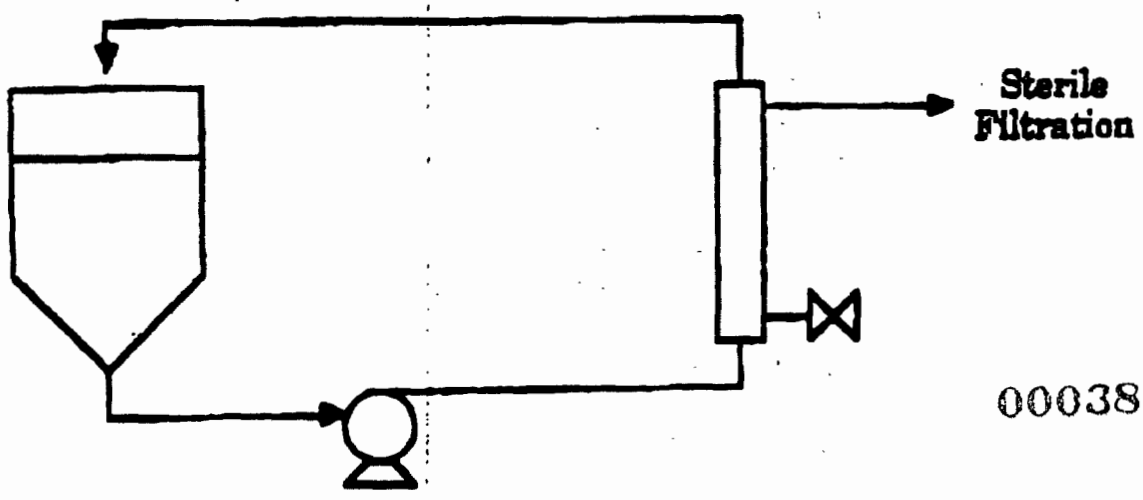
Since the Sterilox element is reusable, the element is depyrogenated at the end of each process cycle with nitric acid and then flushed to pH with pyrogen-free water. Tests at IWT have shown that the permeate exiting a Sterilox element after the recommended cleaning/rinse cycle have less than 0.125 pyrogen EU/ml.

CLEANING PROTOCOL

The Sterilox ceramic membrane filter should be cleaned in a cross-flow mode (see Figure 3). The following cleaning regimen has been demonstrated as being effective in the cleaning of elements exposed to milk products, fermentation broths, and human proteins.

Figure 3

CROSS-FLOW FILTRATION



000383

CERAMIC MEMBRANE CLEANING SYSTEM DESIGN

Cleaning cycles must be run in cross-flow configuration (either on system or on separate cleaning system)

- Recommended cleaning cycle

1. Fresh water rinse (20° C)	10 minutes
2. Recycled NaOH, (2%) @ 60° C	20 minutes
3. Fresh water rinse (20° C)	10 minutes
4. Recycled Nitric Acid (2%) @ 60°C	20 minutes
5. WFI water rinse to pH	10 - 15 minutes

6. If element will not be used immediately, it should be steam sterilized to maintain pyrogen control

000384

ILLINOIS WATER TREATMENT COMPANY

ALUMINA EXTRACTABLE TESTING OF 0.2 μM STERILOX ELEMENTS

Sterilox elements were tested for extraction of alumina when operated in the presence of .2 megohm water, 1.4 megohm water, and hot 2% nitric acid. The permeate was sampled during each run and analyzed with a Perkin Elmer Model 5000 Atomic Absorption Spectrophotometer with graphite furnace at a furnace temperature of 2500°C. The following table lists the analytical results from these tests.

1 hr. to 20 hrs. recirculated .2 megohm water:	Non-Detectable
1 hr. to 18 hrs. recirculated 1.4 megohm water:	Non-Detectable
Nitric Acid (60°C) recirculated for 10 min.:	.18 ppm

Note: AA instrument limit of detection is 10 ppb.

000385

ILLINOIS WATER TREATMENT COMPANY

DETERMINATION OF CLEANING EFFICIENCY OF THE [redacted] MULTI-CHANNEL MEMBRANE ELEMENT

Membralox cross-flow ceramic microporous membrane was penetrated with a Tartrazine dye solution and the subsequent measurement of the degree of elimination of that dye from the element during a conventional cleaning procedure. (Full report in Appendix E)

Since the Membralox multi-channel ceramic elements are sealed at their ends by means of an impregnation of small grain reactive alumina within the porous support and a surface deposit of a continuous layer of silica and calcium-based glaze (Figure 1a & 1b), a pocket is formed at each end of the Membralox element. This pocket is considered the most difficult section of the element to clean. Elimination of a Tartrazine dye from this pocket during a final cleaning methodology is considered proof that the ceramic element can be satisfactorily cleaned for reuse.

I. EQUIPMENT AND METHODS

1.1 Membrane

The study utilized a microfilter multi-channel Membralox membrane 20×10^{-2} meters long. After the test, the membrane was cut into sections approximately 4×10^{-3} meters thick. The location of the membrane sections are indicated in Figure 2.

1.2 Microfiltration Apparatus and Operating Conditions

The membrane was installed in a microfiltration apparatus, the operation of which is illustrated in Figure 3.

Four liters of a 0.5g/liter Tartrazine dye solution was in the apparatus recirculated and subsequently cleaned under the following operating conditions:

- average transmembrane pressure: 0.5 bar
- crossflow circulation velocity: 0.2 m/sec
- ambient temperature except for cleaning acid and caustic which were run @ 60°C
- the filtrate and retentate were recycled except during water rinses

1.3 Dye and Recovery

The dye used was Tartrazine supplied by the Wackherr Company.

The dye concentration in each feed and extraction solution was measured with a spectrophotometer at a wavelength of 429 nm. A standard concentration curve was previously determined with a range of dye standards (5×10^{-2} to 5×10^{-5} g/liter).

000386

The dye was recovered from each section by several 15 minute rinses at 30°C using ultrasound in successive baths (10 ml) of distilled water, NaOH (2%) and nitric acid (2%) until total depletion (defined as no change in dye extraction when checked under the same conditions in the presence of caustic and nitric acid at a concentration of 10%). The total amount of dye contained in each section was then equal to the total of the dye concentration in all the water and chemical rinses.

II. Test Methodology

The element was assembled and installed in the test apparatus (Figure 3) and operated under the conditions indicated in section 1.2 for the filtration of 4 liters of water containing 0.5 g/liter Tartrazine solution. The test duration was 1 hour.

Cleaning was accomplished within the same apparatus by applying the following commonly used cleaning procedure.

- fresh water rinse (20°C)	10 minutes
- recycled 2% NaOH (60°C)	20 minutes
- fresh water rinse (20°C)	10 minutes
- recycled 2% nitric acid (60°C)	10 minutes
- fresh water rinse (20°)	10 minutes

The results listed in Table 1 were obtained by determining the amount of dye left in each membrane section after each cleaning step.

III. CONCLUSION

3.1. Demonstrated that it is possible to recover all the dye which has penetrated the porous membrane environment by a reasonable number of successive water, soda, and nitric acid baths using ultrasound.

3.2. Determined the effect of each cleaning step in a typical cleaning cycle (water - caustic - water - acid - water) on the extraction of dye previously fixed in the porous membrane by means of microfiltration of the test dye solution for a period of one hour.

3.3. Confirmed that less than .01 ug of dye/gram of membrane remains in any part of the porous membrane regardless of its location with respect to the sealed end of the membrane.

000387

SP Sepharose® Ion Exchange Media

000388

Pharmacia Biotech

Safety Data Sheet

Date: 96-01-01

1. Identification of the Substance/Preparation and of the Company/Undertaking

Trade name and synonyms		Manufacturer
SP Sepharose® Big Beads		Pharmacia Biotech AB
Catalogue No.	CAS No Prod	S-751 82 Uppsala, Sweden
17-0657-03, -05, -60	None	Telephone No. +46 18 16 50 00
Description		
Ion exchange chromatography media		

2. Composition/Information on Ingredients

Chemical family			
Alkylsulphonic acid groups covalently bound to highly cross-linked agarose			
Apperance and Odour			
White suspension. A faint odour of ethanol			
Principal hazardous component(s)	CAS - No Comp	Conc. (%)	Symbol/R-phrases
Ethanol	64-17-5	20	R 10

3. Hazards Identification

Routes of entry	Effects of Occupational Overexposure
○ Inhalation ◉ Eye contact ◉ Skin contact ○ Ingestion	Ethanol might have a drying and irritating effect on the skin after repeated or prolonged exposure

4. First-Aid Measures

Inhalation:	Remove from exposure
Eyes:	Wash thoroughly with water for 15 minutes. If discomfort persists, obtain medical attention
Skin:	Wash off with water
Ingestion:	Wash out mouth with water and give water to drink

5. Fire-Fighting Measures

Extinguishing media	
Carbon dioxide, dry chemicals or water spray	
Special fire fighting procedures	Unusual fire and explosive hazards
Use media proper to the primary cause of fire	None

6. Accidental Release Measures

Steps to be taken if material is released or spilled

Wipe up and place in containers for disposal. Floor may be slippery.

7. Handling and Storage

~~000389~~

Precautions to be taken in handling and storage etc.

Store gel suspensions in the presence of an antimicrobial agent e.g. 20% ethanol or in 0.2M sodium acetate

HFK-131 (PES)

000390

KOCH MEMBRANE SYSTEMS
Fluid Systems

Fax Transmittal

Date:	**November 29, 1999**		
TO:	**Ram Nimagudda** ***DMV Nutrionals***	**PHONE:** **FAX:**	**(607) 746-3082** **(607) 746-8245**
FROM:	**Patrick Martin** ***Koch Membrane Systems, Inc.***	**PHONE:** **FAX:**	**(978) 694-7132** **(978) 694-7020**
RE:	**HFK131 Spiral Specifications**		
CC:			

Number of pages including cover sheet: 4

Mr. Nimagudda,

The following is a specification sheet for our HFK131 sanitary spiral. The attached describes the 3.8" diameter, 38" long configuration. We also produce 6" and 8" diameter products in lengths which vary from 33"-38", however the materials of construction are similar.

The membranes are wetted with 70% glycerine prior to shipment. I have attached an MSDS for the glycerine that is used for this process.

I hope the attached information is helpful.

Best Regards,
Koch Membrane Systems

Patrick Martin
Eastern Regional Sales Engineer

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KOCH PRODUCT SPECIFICATION

PART NUMBER:	0741295	REVISION:	2
DESCRIPTION:	KOCH XL™-1000 4336-HFK-131-VYV	DATE:	4/12/98

CONFIGURATION: SPIRAL

COMPONENTS

MEMBRANE:	HFK-131 (PES)
FEED SPACER:	43 MIL POLYPROPYLENE NETTING
OVERWRAP/HOUSING SEAL:	43 MIL POLYPROPYLENE NETTING
PERMEATE TUBE:	POLYSULFONE, FLUSH-CUT
FDA STATUS:	[redacted]

DIMENSIONS/PROPERTIES:

DRAWING NUMBER:	D5238-5064	–
NOMINAL LENGTH:	35.5"	(90 CM)
NOMINAL OUTSIDE DIAMETER:	4.3"	(10.9 CM)
NOMINAL PERMEATE TUBE ID:	0.830"	(2.108 CM)
NUMBER OF LEAVES:	3	–
APPROXIMATE MEMBRANE AREA		
MEAN:	76 ft.²	(7.1 M²)
TYPICAL RANGE:	69-83 ft.²	(6.5-7.7 M²)
WATER FLUX @ 50 PSI, 77°F (BASED ON CLEAN MEMBRANE USING RO WATER)		
MEAN:	65 GFD	(110 LMH)
TYPICAL RANGE:	50-80 GFD	(85-135 LMH)
FEED FLOW vs. PRESSURE DROP: (BASED ON RO WATER AT 77°F)	$Q_{(GPM)} = 6.15\ \Delta P_{(PSI)}^{0.563}$	

RECOMMENDED OPERATING LIMITS (BASED ON MEAN LIFE TARGET OF 18 MONTHS)

MAXIMUM INLET PRESSURE:	140 PSI	(9.7 BAR)
MINIMUM OUTLET PRESSURE:	15 PSI	(1.0 BAR)
MAXIMUM PERMEATE PRESSURE:	5 PSI	(0.3 BAR)
	NO REVERSE PRESSURE	
MAXIMUM FEED-SIDE PRESSURE DROP		
PER SPIRAL:	25 PSI @ 130°F	(1.7 BAR @ 54°C)
PER HOUSING:	66 PSI @ 130°F	(4.5 BAR @ 54°C)
MAXIMUM TEMPERATURE AT pH 6:	130°F (54°C)	
MAXIMUM pH RANGE AT 130°F (54°C)		
CONTINUOUS:	2.5 - 10.5	
SHORT TERM:	1.5 - 11.0	
MAXIMUM CHLORINE (CLEANING):	200 PPM @ pH 10.5-11.0, 130°F (54°C) 30 MINUTES PER DAY TOTAL 0 PPM AT pH LESS THAN 9.5	

000392

PROCTER & GAMBLE
INDUSTRIAL CHEMICALS DIVISION
P.O. BOX 599, CINCINNATI, OHIO 45201

MATERIAL SAFETY DATA SHEET

PROCTER & GAMBLE OPERATOR (513) 921-1100 (24 Hour Emergency Service)

SECTION I IDENTITY

ISSUE DATE 10/88

Trade Names: Superol Glycerine USP, Star Glycerine USP 01.03
Kosher Superol Glycerine USP, Kosher Star Glycerine USP

Chemical Identity: Glycerol derived from animal and vegetable oils (1,2,3-Propanetriol)

CAS Number: 56-81-5

SECTION II PHYSICAL DATA

BOILING POINT	@760 mmHg	Over 550 F	SPECIFIC GRAVITY (H2O=1) @22/22 C	1.2 - 1.3
VAPOR PRESSURE	@72 F	Less than 0.1 mm	PERCENT VOLATILE BY VOLUME (%)	Not known
VAPOR DENSITY (AIR=1)		Not known	EVAPORATION RATE (BuOAc=1)	Not known
SOLUBILITY IN WATER	@72 F	Complete		
APPEARANCE AND ODOR	@72 F	Water white, clear liquid; bland odor; sweet taste		

SECTION III FLAMMABILITY AND EXPLOSIVITY DATA

FLASH POINT (METHOD): Over 390 F (PMCC)

EXPLOSIVE LIMITS LOWER / UPPER: N/A

EXTINGUISHING MEDIA: Use water, CO_2, or dry chemical

SPECIAL FIRE FIGHTING PROCEDURES: None

UNUSUAL FIRE AND EXPLOSION HAZARDS: Contact of glycerine with strong oxidising agents such as Chromium Trioxide, Potassium Chlorate, or Potassium Permanganate may cause an explosion.

SECTION IV HEALTH AND SAFETY DATA

THRESHOLD LIMIT VALUE: Glycerine mist - nuisance particulate 10 mg/m^3

EFFECTS OF OVEREXPOSURE: No harmful effects expected.

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EMERGENCY AND FIRST AID PROCEDURES: None required.

P&G CHEMICALS

GLYCERINE
USP NATURAL, FOOD GRADE

	Analytical Methods	SUPEROL & KOSHER SUPEROL	STAR
% Glycerol (Bauart & Snoddy Tables)	V-F-37a or 37b	99.7 minimum (99.9)	96 minimum (96.3)
Specific Gravity by density meter: @ 25°/25°C	V-F-37a or 37b	1.2613 minimum (1.2618)	1.2517 - 1.2531 (1.2524)
Assay (anhydrous basis)	V-A-27	99-101% (Pass)	99-101% (Pass)
Moisture	ANC015.01	0.3% maximum	4.0% maximum
Color APHA Pt-Co (Hazen) scale	ANC007.01	10 maximum (0)	10 maximum (0)
Residue on Ignition	V-F-9	0.007% or 70 ppm max.	0.007% or 70 ppm max.
Chlorides (as Chlorine)	ANC009.02	0.0010% or 10 ppm max.	0.0010% or 10 ppm max.
Sulfates	ANC010.03	0.002% or 20 ppm max.	0.002% or 20 ppm max.
Heavy metals (as Pb)	ANC016.01	0.0005% or 5 ppm max.	0.0005% or 5 ppm max.
Chlorinated Compounds (as Cl)	V-F-22	0.003% or 30 ppm max.	0.003% or 30 ppm max.
Fatty Acids & Esters	ANC014.01	Not more than 0.3 ml. N/2 NaOH is absorbed by 50 g. of glycerine, which is equivalent to 0.0009% as Na_2O, max. (0.13)	Not more than 0.3 ml. N/2 NaOH is absorbed by 50 g. of glycerine, which is equivalent to 0.0009% as Na_2O, max. (0.12)
Chromatographic Purity	USP 23, 21 Interim Rev.	Individual Impurity ≤ 0.1% Sum Of All Impurities ≤ 1.0%	Individual Impurity ≤ 0.1% Sum Of All Impurities ≤ 1.0%
Identity By GC	USP 23, 21 Interim Rev.	Passes test as glycerine	Passes test as glycerine
Organic Volatile Impurities	USP 23, 21 Interim Rev.	benzene ≤ 100ppm; chloroform ≤ 50ppm; 1,4-dioxane ≤ 100ppm; methylene chloride ≤ 500ppm; trychloroethylene ≤ 100ppm	benzene ≤ 100ppm; chloroform ≤ 50ppm; 1,4-dioxane ≤ 100ppm; methylene chloride ≤ 500ppm; trychloroethylene ≤ 100ppm

CAS No. 56-81-5

000394

Pellicon 2 Filter

000395

MILLIPORE

80 Ashby Rd.
Bedford, MA 01730

Millipore is a registered trademark of Millipore Corporation or an affiliated company.

P35718 Rev. — 1/95

MILLIPORE

CERTIFICATE OF

QUALITY

The purpose of this certificate is to provide precise information on the quality characteristics and acceptance criteria which support the high standards of quality and reliability built into Millipore products.

Millipore certifies that the product described within meets the following criteria.

MILLIPORE

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Pellicon 2 Filter

Catalogue Number: P2HVMPV05
Lot Number: P8AM9124
Membrane Type: Durapore
Membrane Area: 0.5 m^2

Good Manufacturing Practices

This product was manufactured in a Millipore facility which meets or exceeds FDA Device Good Manufacturing Practice standards.

ISO 9000 Quality Standard

This product was manufactured in a Millipore facility whose Quality Management System is approved by an accredited registering body to the appropriate ISO 9000 Quality Systems Standard.

Materials Polyvinylidene fluoride

Membrane: [redacted]
Screens: Polypropylene
Endcap: Polysulfone
Adhesives: Urethane
Toxicity: All parts in fluid paths were tested and meet the criteria of the USP Class VI Biological Tests for Plastics.

100% Integrity Tested in Manufacturing

Each unit must pass the Millipore Integrity Test based on air flow through the fully-wetted membranes of the filter.

Quality Assurance Lot Release Criteria

Every unit is tested by Manufacturing and released by Quality Assurance to the following specifications:

Integrity

Each unit exhibited air flow through fully-wetted membranes less than or equal to 12 cc/min at 10 psig (0.7 bar) inlet pressure.

Pressure Drop

Samples met a maximum pressure drop of less than or equal to 3.5 psi (0.24 bar) at 0.8 gpm (3.0 L/min) average cross flow of clean water.
(For 2.0m^2 Maxi's, note that test is done on 0.5m^2 elements.)

Pellicon is a trademark of Millipore Corporation or an affiliated company. P60094 Rev. — 12/97

Quality Assurance Audit Criteria

This product was designed and manufactured to meet the following specifications. Performance is confirmed by testing on an audit basis.

USP Oxidizable Substances

Effluent was negative after an RO water flush.

Gravimetric Extractables

The extractables level was equal to or less than 1500 mg/m^2 after an RO water flush.

Nicholas Lambo

Nicholas Lambo
Vice President and General Manager,
BioProcess Division

John P. Tuttle
Manager, Worldwide Quality Systems
and Certification, BioProcess Division

MILLIPORE

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Worldwide Product Consistency

Millipore is commited to ensuring consistent processes and products to the industries we support. Our manufacturing processes are designed to manufacture products that consistently meet specifications. Products are consistent not only from lot to lot, but from plant to plant. Before release, all Millipore products are tested to ensure they meet specification requirements. Customers have the assurance that products supplied by one Millipore facility are the same quality and perform the same as those products supplied by any other Millipore site.

Employee Involvement

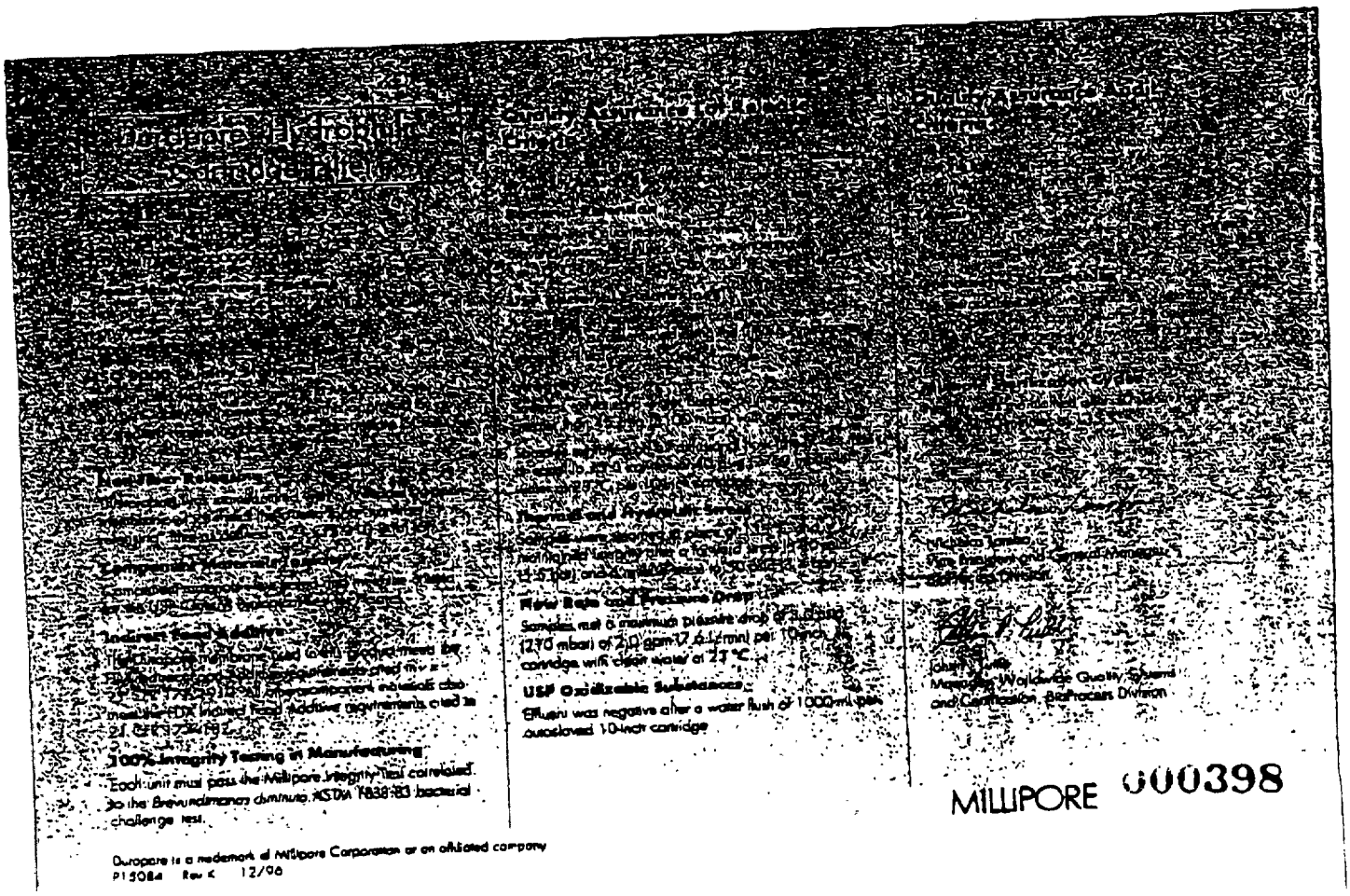
Millipore employees are empowered with responsibility for the quality of our products and services. Quality begins with, and ultimately relies on, the commitment of the people manufacturing the product and providing the service. Training in quality principles assures that Millipore employees keep customer satisfaction as their top priority.

Maintenance of Millipore's Quality System is the responsibility of every employee. The empowerment of our employees is evident in many aspects of our business practices. Every Millipore employee has the authority to shut down a manufacturing line if they believe that changes are needed. Any employee can request changes to our documentation system.

ISO 9000 Registration

Every major Millipore facility worldwide is registered to the applicable ISO 9000 Quality Systems Standard. Our manufacturing facilities and distribution centers are registered to ISO 9002 "Quality Systems—Model for Quality Assurance in Production and Installation." Our product design and development center is registered to ISO 9001 "Quality Systems—Model for Quality Assurance in Design/Development, Production, Installation, and Servicing." These independent certifications of our Quality System are further assurance of Millipore's quality products and services.

000398



Durapore is a trademark of Millipore Corporation or an affiliated company
P15084 Rev K 12/96

Certificates of Quality

Millipore provides its customers with written Certificates of Quality substantiating product properties and performance. Our standard Certificate of Quality is product-specific and verifies conformance to lot release criteria, product audit criteria and in-process testing. The certificate is packaged with our major pharmaceutical product lines. A custom Certificate of Quality typically lists the product's properties and lot release criteria. This certificate is prepared upon customer request for products that do not have a standard Certificate of Quality. The certification can take the place of expensive, difficult or time-consuming incoming inspection testing. [redacted] requirements for validation of new performance.

Notification of Change

At Millipore, we recognize the need for stable process materials in the pharmaceutical industry. Changes in a supplier's product can require revalidation of the pharmaceutical manufacturing process. However, continuous improvement efforts, change in raw materials caused by suppliers, and improvements requested during customer audits may result in necessary changes to our products. A change notification process has been implemented to assure that changes to Millipore products are communicated to our pharmaceutical customers.

Proposed changes are evaluated for their impact prior to implementation. Changes which affect product form, fit, or function are communicated to pharmaceutical customers before implementation, so our customers can properly determine the impact the change may have on their processes. Change notifications include detailed information to judge revalidation requirements and support change in customers' documentation.

Millipore's change notification process is our way of balancing continuous improvement initiatives with the stability requirements of the pharmaceutical industry.

Global Customer Care (GCC)

Global Customer Care (GCC) is an integral part of our Quality System. The computerized GCC system is worldwide in scope and encompasses all manufacturing plants. As part of our procedures, the GCC system's major functions include: continually gathering information on the experiences of our customers with Millipore products, routinely monitoring and reporting those experiences to all levels of management, providing a management system for resolving customer problems or complaints, taking corrective actions where appropriate, and examining the customer information to identify, delineate and report trends to the appropriate functions within Millipore.

The most important function of the GCC system is to support our customers' immediate needs. For instance, replacement product may be needed to keep a customer in operation, or technical assistance may be needed to focus on a specific issue. Once an immediate need is identified, the cause and a solution for the customer inquiry is sought. The solution may involve such groups as manufacturing, engineering, purchasing and R&D. The customer interacts with the GCC system through the local Millipore Application Specialist, Technical Service Representative or Pharmaceutical Field Marketing Manager.

Auditing Quality

Millipore's ultimate goal is to provide products and services that satisfy our customers' needs. To ensure our quality systems are operating as designed, we perform self audits. These audits provide us with ongoing internal communications that lead to improvements in our manufacturing processes.

In addition, Millipore is routinely audited by external agencies such as the U.S. Food and Drug Administration (FDA) for compliance to the Medical Device GMP regulations, and internationally accredited registrars for compliance to the ISO 9000 Quality Systems Standards.

Lastly, you, the customer, are welcome to visit or audit our worldwide manufacturing facilities to observe our Quality Management System in operation.

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APPENDIX C:

Batch Analysis

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DMV

CERTIFICATE OF STANDARDS OF LACTOFERRIN

(Adjusted for GRAS-registration purposes)

	Specification	Typical
Protein (Nx6.38)	min. 93.0 %	95.5 %
Moisture	max. 6.0 %	4.0 %
LF on protein	min. 95.0 %	97.0 %
Ash (550°C)	max. 1.0 %	0.5 %
pH (2%, 20°C)	5.5 - 6.5	6.1
Bulkdensity	-	0.3 g/ml
Solubility:		
in water, 20°C		completely at 2%
transmittance, 2% sol., 600 nm	min. 80 %	82 %
Iron binding:		
spectrophotometric method at 465 nm (on solids)	min. 70 %	76 %
Foreign matter (10 g)		absent
Heavy metals (as lead)	max. 1 mg/kg	< 0.25 mg/kg
Standard plate count	max. 1,000 /g	500 /g
Enterobacteriaceae	max. 10 /g	<10 /g
Yeast	max. 10 /g	<10 /g
Moulds	max. 10 /g	<10 /g
Staphylococcus aureus 2x1 g	negative	negative
Salmonella	neg. in 50 g	neg. in 50 g

Remarks

1. The analytical data mentioned in this "Certificate of Standards" refer to internationally accepted methods (IDF, ISO, AOAC) and are available on special request.
2. Microbiological specifications are based on EC food legislation including EC 92/46 (hygiene guidelines)
3. Based on quality of raw materials, equipment, process and GMP's we further guarantee the absence of harmfull amounts of pathogenic bacteria like Lysteria monocytogenes, Cl. perfringens, Brucellosis, Yersinia and E.coli.

Quality Assurance Manager

C.J.M. Habraken

Date of issue : August 1999
Supersedes issue date : May 1998

000401

000401

DMV INTERNATIONAL

Contaminants
(Adjusted for GRAS-registration purposen)

Product : LACTOFERRINE
Issue date : August 1999
Expiry date : August 2003

	Typical value	Specification	Unit
Pb (lead)	< 0.3	< 0.5	mg/kg
As (arsenic)	< 0.3	< 1.0	mg/kg
Cd (cadmium)	< 0.03	< 0.05	mg/kg
Hg (mercury)	< 0.03	< 0.05	mg/kg
Cu (copper)	< 1.5	< 2.0	mg/kg
PCB's (total [1])	< 0.1	< 0.1	mg/kg
Pesticides (total [2])	< 0.1	< 0.1	mg/kg
Antibiotics	< 0.003	< 0.005	IU/ml
Aflatoxine M1	< 0.3	< 0.5	µg/kg
Radioactivity ($Cs_{134} + Cs_{137}$)	< 2	< 5	Bq/kg

[1] PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153, PCB 180
[2] Dieldrin, heptachlor epoxide, hexachlorobenzene, α -hexachlorocyclohexane, ß-hexachlorocyclohexane, lindane, DDT-total

All levels are far below applicable food legislation requirements

C.J.M. Habraken
Quality Assurance Manager

Virtually all our products are permitted in accordance with the Food Legislation in many countries. We do, nevertheless advice customers to check the appropriate Food Legislation with regard to the application. The details given here are intended merely for information purposes and are in no way legally binding. Consequently we accept no responsibility, in the broadest sense of the word, for damage that may result from application of this information. Furthermore, this information does not constitute permission to infringe patent and licence rights.

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DMV International
division of Campina Melkunie bv
NCB-laan 80 P.O. Box 13,
5460 BA Veghel - The Netherlands
Telephone +31 0413 372222
Telefax +31 0413 343695

000402

000402

DMV INTERNATIONAL

Contaminants

Product : LACTOFERRINE
Issue date : April 1998
Expiry date : October 2002

	Typical value	Unit
Pb (lead)	< 0.3	mg/kg
As (arsenic)	< 0.3	mg/kg
Cd (cadmium)	< 0.03	mg/kg
Hg (mercury)	< 0.03	mg/kg
Cu (copper)	< 1.5	mg/kg
PCB's (total [1])	< 0.1	mg/kg
Pesticides (total [2])	< 0.1	mg/kg
Antibiotics	< 0.003	IU/ml
Aflatoxine M1	< 0.3	µg/kg
Radioactivity (Cs 134 + Cs 137)	< 2	Bq/kg

[1] PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153, PCB 180
[2] Dieldrin, heptachlor epoxide, hexachlorobenzene, α -hexachlorocyclohexane, ß-hexachlorocyclohexane, lindane, DDT-total

All levels are far below applicable food legislation requirements

C.J.M. Habraken
Quality Assurance Manager

000403

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DMV International,
division of Campina Melkunie bv
NCB-laan 80, P.O. Box 13,
5460 BA Veghel - The Netherlands
Telephone +31 (0)413 37 22 22
Telefax +31 (0)413 34 36 95

000403

DMV INTERNATIONAL

MATERIALS SAFETY DATA SHEET

Product	:	Lactoferrin
Description	:	Lactoferrin is a purified and spray-dried protein fraction from milk

Physical data

Appearance and odor	:	Salmon-coloured powder, neutral
Solubility in water	:	About 2 g/100 ml at 20°C

Fire and explosion hazard data

Ignition temperature	:	not available
Selfignition temperature	:	not available
Special fire fighting procedure	:	None

Health hazard data	:	Lactoferrin is a food ingredient used in very low concentrations, though avoid extra ingestion and inhalation.

Spill or leak procedures

In the case material is released or spilled	:	Sweep, vacuum or waste with water. Avoid dust formation.

Storage conditions	:	Store at below 8°C.

Special precautions :

- Avoid inhalation and skin contact.
- During storage keep packingbags unopened to avoid contamination and growth of fungi.
- When heated to decomposition it emits acrid smoke and fumes.

DMV International

F.M. van Haaren
Food Law Department

Date of issue: February 1995
Supersedes all previous editions (not modified)

000404

Virtually all our products are permitted in accordance with the Food Legislation in many countries. We do, nevertheless, advice customers to check the appropriate Food Legislation with regard to the application. The details given here are intended merely for information purposes and are in no way legally binding. Consequently we accept no responsibility, in the broadest sense of the word, for damage that may result from application of this

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division of Campina Melkunie bv
HCB-laan 80, P.O. Box 13,
5460 BA Veghel - The Netherlands
Telephone +31 (0)413 37 22 22
Telefax +31 (0)413 34 36 95

000404

DMV INTERNATIONAL

To whom it concerns

AvD 18 -08-1998.

DECLARATION

Herewith we, DMV-International, declare that our product Lactoferrin is produced according to Good Manufacturing Practices and meets the requirements of the EC and Dutch Food Legislation and is as an ingredient fit for human consumption.
The during manufacturing used chemicals are at [redacted] quality.

DMV International

T.v.Doom
Quality Assurance

000405

Virtually all our products are permitted in accordance with the Food Legislation in many countries. We do, nevertheless, advice customers to check the appropriate Food Legislation with regard to the application. The details given here are intended merely for information purposes and are in no way legally binding. Consequently we accept no responsibility, in the broadest sense of the word, for damage that may result from application of this

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division of Campina Melkunie bv
NCB-laan 80, PO Box 13,
5460 BA Veghel - The Netherlands
telephone +31 (0)413 37 22 22
Telefax +31 (0)413 34 36 95

000405

DMV INTERNATIONAL

To whom it concerns

RH/IS/d97071 21-04-1997

DECLARATION

Herewith we, DMV International, declare that our product Lactoferrin has a shelflife of at least one year at 20 °C and two years at 8 °C.

This shelflife is applicable in the unopened, original packaging stored at moderate (max. 75%) relative humidity.

Kind regards,

DMV International ,

K. Haarsten
Quality Assurance Manager

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Virtually all our products are permitted in accordance with the Food Legislation in many countries. We do, nevertheless, advice customers to check the appropriate Food Legislation with regard to the application. The details given here are intended merely for information purposes and are in no way legally binding. Consequently we accept no responsibility, in the broadest sense of the word, for damage that may result from application of this

DMV International,
division of Campina Melkunie bv
NCB-laan 80, P.O. Box 13,
5460 BA Veghel - The Netherlands
Telephone +31 (0)413 37 22 22
Telefax +31 (0)413 34 36 95

1 Afzender / Consignor / Expéditeur / Remitente

DMV International, Nutritionals

Veghel

Nr. 156224.
23090

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EUROPESE GEMEENSCHAP
EUROPEAN COMMUNITY
COMMUNAUTÉ EUROPÉENNE
COMUNIDAD EUROPEA

CERTIFICAAT VAN OORSPRONG
CERTIFICATE OF ORIGIN
CERTIFICAT D'ORIGINE
CERTIFICADO DE ORIGEN

2 Geadresseerde / Consignee / Destinataire / Destinatario

DMV International, Nutritionals
1712 Deltown Plaza
FRASER, New York
USA

3 Land van oorsprong / Country of origin / Pays d'origine / País de origen

EUROPEAN COMMUNITY
THE NETHERLANDS

4 Gegevens in verband met het vervoer (facultatief) / Transport details (optional) / Informations relatives au transport (mention facultative) / Expedicion

5 Opmerkingen / Remarks / Remarques / Observaciones

6 Volgnummer; merken, nummers, aantal en aard van de colli; omschrijving van de goederen

Lactoferrin,
Whey Protein Fraction

7 Hoeveelheid

kg

000407

000407

8 ONDERGETEKENDE AUTORITEIT VERKLAART DAT DE HIERBOVEN VERMELDE GOEDEREN VAN OORSPRONG ZIJN UIT HET IN VAK 3 GENOEMDE LAND
THE UNDERSIGNED AUTHORITY CERTIFIES THAT THE GOODS DESCRIBED ABOVE ORIGINATE IN THE COUNTRY SHOWN IN BOX 3
L'AUTORITE SOUSSIGNEE CERTIFIE QUE LES MARCHANDISES DÉSIGNÉES CI-DESSUS SONT ORIGINAIRES DU PAYS FIGURANT DANS LA CASE No. 3
LA AUTORIDAD INFRASCRITA CERTIFICA QUE LAS MERCANCIAS DESIGNADAS SON ORIGINARIAS DEL PAIS INDICADO EN LA CASILLA No. 3

CHA
KAA

KAMER VAN KOOP
OOST-BRABANT
'S-HERTOGENBOS

Plaats en datum van afgifte; aanduiding, handtekening en stempel van de bevoegde autoriteit
Place and date of issue; name, signature and stamp of competent authority
Lieu et date de délivrance; désignation, signature et cachet de l'autorité compétente
Lugar y fecha de expedicion; designacion, firma y sello de la autoridad competente

1 Afzender / Consignor / Expéditeur / Remitente

DMV International, Nutritionals

Veghel

Nr. T 562214
23090

ORIGINEEL/
ORIGINAL

EUROPESE GEMEENSCHAP
EUROPEAN COMMUNITY
COMMUNAUTÉ EUROPÉENNE
COMUNIDAD EUROPEA

CERTIFICAAT VAN OORSPRONG
CERTIFICATE OF ORIGIN
CERTIFICAT D'ORIGINE
CERTIFICADO DE ORIGEN

2 Geadresseerde / Consignee / Destinataire / Destinatario

DMV International, Nutritionals
1712 Deltown Plaza
FRASER, New York
USA

3 Land van oorsprong / Country of origin / Pays d'origine / País de origen

EUROPEAN COMMUNITY THE NETHERLANDS

4 Gegevens in verband met het vervoer (facultatief) / Transport details (optional) / Informations relatives au transport (mention facultative) / Expedición

5 Opmerkingen / Remarks / Remarques / Observaciones

6 Volgnummer; merken, nummers, aantal en aard van de colli; omschrijving van de goederen

Lactoferrin,
Whey Protein Fraction

7 Hoeveelheid

kg

000408

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L'AUTORITE SOUSSIGNÉE CERTIFIE QUE LES MARCHANDISES DÉSIGNÉES CI-DESSUS SONT ORIGINAIRES DU PAYS FIGURANT DANS LA CASE No. 3
LA AUTORIDAD INFRASCRITA CERTIFICA QUE LAS MERCANCIAS DESIGNADAS SON ORIGINARIAS DEL PAIS INDICADO EN LA CASILLA No. 3

000408

CHAMBER OF COMMERCE AND INDUSTRY
KAMER VAN KOOPHANDEL EN FABRIEKEN
VOOR OOST-BRABANT (NEDERLAND)

Plaats en datum van afgifte; aanduiding, handtekening en stempel van de bevoegde autoriteit
Place and date of issue; name, signature and stamp of competent authority
Lieu et date de délivrance; désignation, signature et cachet de l'autorité compétente
Lugar y fecha de expedición; designación, firma y sello de la autoridad competente

KAMER VAN KOOPHANDEL
OOST-BRABANT
'S-HERTOGENBOSCH

DMV INTERNATIONAL

Date: October 25, 1999
Ref: RH/MU/99068

To whom it concerns

Statement on production codes Lactoferrin.

Herewith we, DMV International, declare that product codenumbers 4053263, 4053726, 4054297 mentioned in COKZ document CVL 92006 refer to production batches of Lactoferrin manufactured by DMV International.

Kind regards,

DMV International
C.J.M. Habraken
QA Manager

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Although our products are permitted in accordance with the Food Legislation in many countries. We do nevertheless advice customers to check the appropriate Food Legislation with regard to the application. The data given here are intended merely for information purposes and are in no way legally binding. Consequently we accept no responsibility in the broadest sense of the word for damage that may result from application of this information. Furthermore, this information does not constitute permission to infringe patent and licence rights.

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DMV International
Division of Campina Melkunie bv
NCB- 22-40 P.O. Box 3
5460 BA Veghel The Netherlands
Telephone +31 413 372222
Telefax +31 413 343645

(0)413-354655 Ablert

Original

CVL92006

Certificate

CERTIFICATE

Centraal Orgaan voor
Kwaliteitsaangelegenheden
in de Zuivel

Netherlands controlling authority for milk and milk products under government supervision
PO Box 250, 3830 AG Leusden, The Netherlands
Tel. (+31)33-4965696, fax (+31)33-4940674, E-mail. boc@cokz nl

We, the Netherlands controlling authority for milk and milk products (COKZ), hereby certify
- that on 28-08-1999 we received from Messrs. DMV International, Veghel / the Netherlands (a) sample(s) indicated as powder of Dutch origin;
- that we analysed the sample(s) chemically and physically according to the undermentioned method(s) of analysis indicated by COKZ A-code(s), with the following result(s):

COKZ-code / analysis aspect		lab. sample number/sample indication/ result of examination		
		E15000 4053263	E15001 4053726	E15002 4054297
A325	- Listeria monocytogenes in 1 g	not detectable	not detectable	not detectable
A307	- Clostridium perfringens in 1 g	not detectable	not detectable	not detectable
A313	- E. coli in 1 g	not detectable	not detectable	not detectable
	- Brucellosis in 1 g	not detectable	not detectable	not detectable
	- Yersinia enterocolytica in 1 g	not detectable	not detectable	not detectable

----------TEXT END----------

Leusden, 13-10-99
On behalf of the director of the
NETHERLANDS CONTROLLING AUTHORITY
FOR MILK AND MILK PRODUCTS,

the proxy

K. Niemeijer

Netherlands Controlling Authority for Milk and Milk Products COKZ

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APPENDIX D:

Milk and Milk Products Used to Estimate Consumption of Bovine Lactoferrin

000411

Milk and Milk Products

CATEGORY	FOODCODE	FOODNAME
Cream	12100100	CREAM, FLUID, NS AS TO LIGHT, HEAVY OR HALF & HALF
Cream	12110100	CREAM, LIGHT, FLUID (INCL COFFEE CRM, TABLE CREAM)
Cream	12110300	CREAM, LIGHT, WHIPPED, UNSWEETENED
Cream	12120100	CREAM, HALF & HALF
Cream	12130100	CREAM, HEAVY, FLUID
Cream	12140000	CREAM, HEAVY, WHIPPED, SWEETENED
Cream	12140100	CREAM, WHIPPED, PRESSURIZED CONTAINER
Fluid Milk	11100000	MILK, NFS
Fluid Milk	11111000	MILK, COW'S, FLUID, WHOLE
Fluid Milk	11111160	MILK, CALCIUM FORTIFIED, COW'S, FLUID, 1% FAT
Fluid Milk	11111170	MILK, CALCIUM FORTIFIED, SKIM/NONFAT, COW, FLUID
Fluid Milk	11112000	MILK, COW'S, FLUID, LOWFAT, NS AS TO % FAT
Fluid Milk	11112110	MILK, COW'S, FLUID, 2% FAT
Fluid Milk	11112120	MILK, COW'S, FLUID, ACIDOPHILUS, 1% FAT
Fluid Milk	11112130	MILK, COW'S, FLUID, ACIDOPHILUS, 2% FAT
Fluid Milk	11112210	MILK, COW'S, FLUID, 1% FAT
Fluid Milk	11113000	MILK, COW'S, FLUID, SKIM OR NONFAT
Fluid Milk	11114300	MILK, LOW LACTOSE, 1% FAT
Fluid Milk	11114310	MILK, LOW LACTOSE, 1% FAT, FORTIFIED WITH CALCIUM
Fluid Milk	11114320	MILK, LOW LACTOSE, NONFAT
Fluid Milk	11114321	MILK, LOW LACTOSE, NONFAT, W/ CALCIUM
Fluid Milk	11114330	MILK, COW'S FL LACTOSE REDUCED 2% FAT (LACTAID)
Fluid Milk	11115000	BUTTERMILK, FLUID (INCLUDE KEFIR MILK)
Fluid Milk	11115200	BUTTERMILK, FLUID, 2% FAT
Fluid Milk	11116000	MILK, GOAT'S, FLUID, WHOLE
Fluid Milk	11120000	MILK, DRY, RECONSTITUTED, NFS
Fluid Milk	11121100	MILK, DRY, RECONSTITUTED, WHOLE
Fluid Milk	11121210	MILK, DRY, RECONSTITUTED, LOWFAT
Fluid Milk	11121300	MILK, DRY, RECONSTITUTED, NONFAT
Fluid Milk	11511000	MILK, CHOCOLATE, NFS
Fluid Milk	11511100	MILK, CHOCOLATE, WHOLE MILK BASED
Fluid Milk	11511200	MILK, CHOCOLATE, LOWFAT MILK BASED
Fluid Milk	11511300	MILK, CHOCOLATE, SKIM MILK BASED
Fluid Milk	11512000	COCOA,HOT CHOCOLATE,NOT FROM DRY MIX, W/WHOLE MILK
Fluid Milk	11512500	HOT CHOCOLATE DRINK W/ EVAP MILK, PUERTO RICAN
Fluid Milk	11513000	COCOA & SUGAR MIXTURE, MILK ADDED, NS TYPE MILK
Fluid Milk	11513100	COCOA & SUGAR MIXTURE, WHOLE MILK ADDED
Fluid Milk	11513200	COCOA & SUGAR MIXTURE, LOWFAT MILK ADDED
Fluid Milk	11513300	COCOA & SUGAR MIXTURE, SKIM MILK ADDED
Fluid Milk	11513400	CHOCOLATE SYRUP MILK ADDED, NS AS TO TYPE OF MILK
Fluid Milk	11513500	CHOCOLATE SYRUP, WHOLE MILK ADDED
Fluid Milk	11513600	CHOCOLATE SYRUP, LOWFAT MILK ADDED
Fluid Milk	11513700	CHOCOLATE SYRUP, SKIM MILK ADDED
Fluid Milk	11519000	MILK BEVERAGE, NOT CHOCOLATE, W/ WHOLE MILK
Fluid Milk	11519050	MILK, NOT CHOCOLATE, WHOLE MILK BASED
Fluid Milk	11520000	MILK, MALTED, UNFORTIFIED, FLAVOR NS
Fluid Milk	11525000	MILK,MALTED,FORTIFIED,NATURAL FLAVOR (INCL OVALTINE
Fluid Milk	11526000	MILK, MALTED, FORTIFIED, CHOCOLATE (INCL OVALTINE)

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Milk and Milk Products

CATEGORY	FOODCODE	FOODNAME
Fluid Milk	11527000	MILK, MALTED, FORTIFIED, (INCL OVALTINE)
Fluid Milk	11541000	MILK SHAKE, NS AS TO FLAVOR OR TYPE
Fluid Milk	11541110	MILK SHAKE, HOMEMADE OR FOUNTAIN-TYPE, CHOCOLATE
Fluid Milk	11541120	MILK SHAKE, HOMEMADE/FOUNTAIN-TYPE, NOT CHOCOLATE
Fluid Milk	11541400	MILK SHAKE WITH MALT (INCL MALTED MILK W/ICE CREAM)
Fluid Milk	11541500	MILK SHAKE, MADE W/ SKIM MILK, CHOCOLATE
Fluid Milk	11541510	MILK SHAKE,MADE W/ SKIM MILK, NOT CHOCOLATE
Fluid Milk	11542100	CARRY-OUT MILK SHAKE, CHOCOLATE
Fluid Milk	11542200	CARRY-OUT MILK SHAKE, NOT CHOCOLATE
Fluid Milk	11551050	MILK FRUIT DRINK (INCL LICUADO)
Fluid Milk	13110000	ICE CREAM, NFS
Fluid Milk	13110100	ICE CREAM, REGULAR, NOT CHOCOLATE
Fluid Milk	13110110	ICE CREAM, REGULAR, CHOCOLATE
Fluid Milk	13110120	ICE CREAM, RICH, FLAVORS OTHER THAN CHOCOLATE
Fluid Milk	13110130	ICE CREAM, RICH, CHOCOLATE
Fluid Milk	13110200	ICE CREAM, SOFT SERVE, NOT CHOCOLATE
Fluid Milk	13110210	ICE CREAM, SOFT SERVE, CHOCOLATE
Fluid Milk	13110220	ICE CREAM, SOFT SERVE, NS AS TO FLAVOR
Fluid Milk	13200110	PUDDING, NFS
Fluid Milk	13210220	PUDDING, CHOCOLATE, RTE, NS FROM DRY MIX OR CANNED
Fluid Milk	13210250	PUDDING, CHOC, RTE, LO CAL, W/ ART SWTNER, NS DRY/CA
Fluid Milk	13210280	PUDDING, NOT CHOC, RTE, NS FROM DRY MIX OR CANNED
Fluid Milk	13210290	PUDDING, NOT CHOC, RTE, LO CAL, W/ ART SWTNER
Fluid Milk	13210520	PUDDING, TAPIOCA,MADE FROM DRY MIX,MADE W/ MILK
Fluid Milk	13210530	PUDDING, TAPIOCA,CHOCOLATE,MADE W/ MILK
Fluid Milk	13210610	PUDDING, COCONUT
Fluid Milk	13220110	PUDDING,NOT CHOCOLATE,PREPARED FROM DRY MIX,MILK ADD
Fluid Milk	13220120	PUDDING,CHOCOLATE,PREPARED FROM DRY MIX,MILK ADDED
Fluid Milk	13220210	PUDDING,NOT CHOC,FROM DRY,LOW CAL,ARTIFICIAL SWEET,W
Fluid Milk	13220220	PUDDING,CHOC,FROM DRY,LOW CAL,ARTIFICIAL SWEET,MILK
Fluid Milk	13230120	PUDDING,CANNED,LOW CAL,W/ARTIFICIAL SWTNR,NOT CHOC
Fluid Milk	13230130	PUDDING, CANNED, CHOCOLATE
Fluid Milk	13230140	PUDDING, CANNED, LO CAL, W/ ART SWTNER, CHOC
Fluid Milk	13230200	PUDDING, CANNED, CHOC & NON-CHOC FLAVORS COMBINED
Fluid Milk	11410000	YOGURT, NS AS TO TYPE OF MILK/FLAVOR
Fluid Milk	11411010	YOGURT, PLAIN, NS AS TO TYPE OF MILK
Fluid Milk	11411100	YOGURT, PLAIN, WHOLE MILK
Fluid Milk	11411200	YOGURT, PLAIN, LOWFAT MILK
Fluid Milk	11411300	YOGURT, PLAIN, NONFAT MILK
Fluid Milk	11420000	YOGURT, VANILLA, LEMON, COFFEE, NS AS TO MILK TYPE
Fluid Milk	11421000	YOGURT, VANILLA, LEMON, COFFEE, WHOLE MILK
Fluid Milk	11422000	YOGURT, VANILLA, LEMON, COFFEE, LOWFAT MILK
Fluid Milk	11423000	YOGURT, VANILLA, LEMON, COFFEE, NONFAT MILK
Fluid Milk	11424000	YOGURT, VANILLA, LEMON, COFFEE, NONFAT MILK, LOW CAL
Fluid Milk	11425000	YOGURT, CHOCOLATE, NS AS TO TYPE OF MILK
Fluid Milk	11426000	YOGURT, CHOCOLATE, WHOLE MILK
Fluid Milk	11427000	YOGURT, CHOCOLATE, NONFAT MILK
Fluid Milk	11430000	YOGURT, FRUIT VARIETY, NS AS TO MILK TYPE

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CATEGORY	FOODCODE	FOODNAME
Fluid Milk	11431000	YOGURT, FRUIT VARIETY, WHOLE MILK
Fluid Milk	11432000	YOGURT, FRUIT VARIETY, LOWFAT MILK
Fluid Milk	11433000	YOGURT, FRUIT VARIETY, NONFAT MILK
Fluid Milk	11433500	YOGURT, FRUITED, NONFAT MILK, LOW CAL SWEETENER
Fluid Milk	11444000	YOGURT, FRUIT & NUTS, NS AS TO TYPE OF MILK
Fluid Milk	11445000	YOGURT, FRUIT & NUTS, LOWFAT MILK
Fluid Milk	11459990	YOGURT, FROZEN, NS AS TO FLAVOR, NS TO TYPE OF MILK
Fluid Milk	11460000	YOGURT, FROZEN, NOT CHOCOLATE, TYPE OF MILK NS
Fluid Milk	11460100	YOGURT, FROZEN, CHOCOLATE, TYPE OF MILK NS
Fluid Milk	11460150	YOGURT, FROZEN, NS AS TO FLAVOR, LOWFAT MILK
Fluid Milk	11460160	YOGURT, FROZEN, CHOCOLATE, LOWFAT MILK
Fluid Milk	11460170	YOGURT, FROZEN, NOT CHOCOLATE, LOWFAT MILK
Fluid Milk	11460190	YOGURT, FROZEN, NS AS TO FLAVOR, NONFAT MILK
Fluid Milk	11460200	YOGURT, FROZEN, CHOCOLATE, NONFAT MILK
Fluid Milk	11460250	YOGURT,FROZEN,NOT CHOCOLATE,W/ SORBET/SORBET-COATED
Fluid Milk	11460300	YOGURT, FROZEN, NOT CHOCOLATE, NONFAT MILK
Fluid Milk	11460400	YOGURT,FRZ,CHOCOLATE,NONFAT MILK,W/ LOW-CAL SWEET
Fluid Milk	11460410	YOGURT,FRZ,NOT CHOC,NONFAT MILK,W/ LOW-CAL SWEET
Fluid Milk	11460420	YOGURT, FROZEN, NS AS TO FLAVOR, WHOLE MILK
Fluid Milk	11460430	YOGURT, FROZEN, CHOCOLATE, WHOLE MILK
Fluid Milk	11460440	YOGURT, FROZEN, NOT CHOCOLATE, WHOLE MILK
Fluid Milk	11461000	YOGURT, FROZEN, CHOCOLATE-COATED
Fluid Milk	11461100	YOGURT, FROZEN, CAROB-COATED
Fluid Milk	11461200	YOGURT, FROZEN, SANDWICH
Fluid Milk	11461250	YOGURT, FROZEN, CONE, CHOCOLATE
Fluid Milk	11461260	YOGURT, FROZEN, CONE, NOT CHOCOLATE
Fluid Milk	11461270	YOGURT, FROZEN, CONE, NOT CHOCOLATE, LOWFAT MILK
Fluid Milk	11461280	YOGURT, FROZ, CONE, CHOCOLATE, LOWFAT MILK

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Infant Formula

CATEGORY	FOODCODE	FOODNAME
Infant Formula	11710000	INFANT FORMULA, NFS
Infant Formula	11710050	ALIMENTUM, W/ IRON, INFANT FORMULA, NS AS TO FORM
Infant Formula	11710051	ALIMENTUM, W/ IRON, INFANT FORMULA, READY-TO-FEED
Infant Formula	11710100	ENFAMIL, LOW IRON, INFANT FORMULA, NS AS TO FORM
Infant Formula	11710101	ENFAMIL, LOW IRON, INFANT FORMULA, READY-TO-FEED
Infant Formula	11710102	ENFAMIL, LOW IRON, INFANT FORMULA, FROM LIQUID CONCE
Infant Formula	11710103	ENFAMIL, LOW IRON, INFANT FORMULA, PREPARED FROM POW
Infant Formula	11710110	GERBER, W/ IRON, INFANT FORMULA, NS AS TO FORM
Infant Formula	11710112	GERBER, WITH IRON, INFANT FORMULA, FROM LIQUID CONCE
Infant Formula	11710113	GERBER, WITH IRON, INFANT FORMULA, PREPARED FROM POW
Infant Formula	11710123	GERBER, LOW IRON, INFANT FORMULA, PREPARED FROM POWD
Infant Formula	11710200	SIMILAC, LOW IRON, INFANT FORMULA, NS AS TO FORM
Infant Formula	11710201	SIMILAC, LOW IRON, INFANT FORMULA, READY-TO-FEED
Infant Formula	11710202	SIMILAC, LOW IRON, INFANT FORMULA, FROM LIQUID CONCE
Infant Formula	11710203	SIMILAC, LOW IRON, INFANT FORMULA, PREPARED FROM POW
Infant Formula	11710400	SIMILAC W/ IRON, INFANT FORMULA, NS AS TO FORM
Infant Formula	11710401	SIMILAC W/ IRON, INFANT FORMULA, READY-TO-FEED
Infant Formula	11710402	SIMILAC W/ IRON, INFANT FORMULA, FROM LIQUID CONCENT
Infant Formula	11710403	SIMILAC W/ IRON, INFANT FORMULA, PREPARED FROM POWDE
Infant Formula	11710450	SIMILAC SPECIAL CARE 24, IRON, INFANT FORMULA, NS FO
Infant Formula	11710461	SIMILAC, TODDLER'S BEST, IRON, INFANT FORMULA, READY
Infant Formula	11710500	SMA, W/ IRON, INFANT FORMULA, NS AS TO FORM
Infant Formula	11710501	SMA, W/ IRON, INFANT FORMULA, READY-TO-FEED
Infant Formula	11710502	SMA, W/ IRON, INFANT FORMULA, FROM LIQUID CONCENTRAT
Infant Formula	11710503	SMA, W/ IRON, INFANT FORMULA, PREPARED FROM POWDER
Infant Formula	11710551	SMA, LOW IRON, INFANT FORMULA, READY-TO-FEED
Infant Formula	11710552	SMA, LOW IRON, INFANT FORMULA, FROM LIQUID CONCENTRA
Infant Formula	11710553	SMA, LOW IRON, INFANT FORMULA, PREPARED FROM POWDER
Infant Formula	11710600	ENFAMIL W/ IRON, INFANT FORMULA, NS AS TO FORM
Infant Formula	11710601	ENFAMIL W/ IRON, INFANT FORMULA, READY-TO-FEED
Infant Formula	11710602	ENFAMIL W/ IRON, INFANT FORMULA, FROM LIQUID CONCENT
Infant Formula	11710603	ENFAMIL W/ IRON, INFANT FORMULA, PREPARED FROM POWDE
Infant Formula	11710711	LACTOFREE, W/ IRON, INFANT FORMULA, READY-TO-FEED
Infant Formula	11710712	LACTOFREE, W/ IRON, INFANT FORMULA, FROM LIQUID CONC
Infant Formula	11710713	LACTOFREE, W/ IRON, INFANT FORMULA, FROM POWDER
Infant Formula	11710801	PEDIASURE, W/ IRON, INFANT FORMULA, READY-TO-FEED
Infant Formula	11710900	CARNATION GOOD START, IRON, INFANT FORMULA, NS FORM
Infant Formula	11710901	CARNATION GOOD START, W/ IRON, INFANT FORMULA, READY
Infant Formula	11710902	CARNATION GOOD START,W/IRON,INFANT FORMULA,LIQUID CO
Infant Formula	11710903	CARNATION GOOD START, W/ IRON, INFANT FORMULA, FROM
Infant Formula	11710951	CARNATION FOLLOW-UP,W/ IRON,INFANT FOR FORMULA,READY
Infant Formula	11710952	CARNATION FOLLOW-UP,W/IRON,INFANT FORMULA,LIQUID CON
Infant Formula	11710953	CARNATION FOLLOW-UP, W/ IRON, INFANT FORMULA, FR POW
Infant Formula	11720051	GERBER SOY, W/ IRON, INFANT FORMULA, READY-TO-FEED
Infant Formula	11720200	NURSOY, W/ IRON, INFANT FORMULA, NS AS TO FORM
Infant Formula	11720201	NURSOY, W/ IRON, INFANT FORMULA, READY-TO-FEED
Infant Formula	11720202	NURSOY, W/ IRON, INFANT FORMULA, FROM LIQUID CONCENT
Infant Formula	11720203	NURSOY, W/ IRON, INFANT FORMULA, PREPARED FROM POWDE
Infant Formula	11720300	PROSOBEE, W/ IRON, INFANT FORMULA, NS AS TO FORM
Infant Formula	11720301	PROSOBEE, W/ IRON, INFANT FORMULA, READY-TO-FEED
Infant Formula	11720302	PROSOBEE, W/ IRON, INFANT FORMULA, FROM LIQUID CONCE

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Infant Formula

CATEGORY	FOODCODE	FOODNAME
Infant Formula	11720303	PROSOBEE, W/ IRON, INFANT FORMULA, PREPARED FROM POW
Infant Formula	11720400	ISOMIL, W/ IRON, INFANT FORMULA, NS AS TO FORM
Infant Formula	11720401	ISOMIL, W/ IRON, INFANT FORMULA, READY-TO-FEED
Infant Formula	11720402	ISOMIL, W/ IRON, INFANT FORMULA, FROM LIQUID CONCENT
Infant Formula	11720403	ISOMIL, W/ IRON, INFANT FORMULA, PREPARED FROM POWDE
Infant Formula	11720502	SOYALAC, W/ IRON, INFANT FORMULA, FROM LIQUID CONCEN
Infant Formula	11740300	NUTRAMIGEN, W/ IRON, INFANT FORMULA, NS AS TO FORM
Infant Formula	11740301	NUTRAMIGEN, W/ IRON, INFANT FORMULA, READY-TO-FEED
Infant Formula	11740302	NUTRAMIGEN, W/ IRON, INFANT FORMULA, FR LIQUID CONC
Infant Formula	11740303	NUTRAMIGEN, W/ IRON, INFANT FORMULA, PREPARED FROM P
Infant Formula	11740500	SIMILAC PM 60/40, LOW IRON, INFANT FORMULA, NS FORM

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Meal Replacements

CATEGORY	SUBCATEGORY	FOODCODE	FOODNAME
Meal replacements	fluid	11611000	INSTANT BREAKFAST, FLUID, CANNED
Meal replacements	fluid	11612000	INSTANT BREAKFAST, POWDER, MILK ADDED
Meal replacements	fluid	11613000	INSTANT BFAST,PWDR,SWT W/ LO CAL SWT, MILK ADDED
Meal replacements	fluid	11621000	DIET BEVERAGE, LIQUID, CANNED
Meal replacements	fluid	11623000	MEAL SUPPLEMENT / REPLACEMENT,PREPARED,RTD
Meal replacements	fluid	11631000	HIGH CALORIE BEV, CANNED OR POWDERED, RECONSTITUTED
Meal replacements	fluid	11641000	MEAL REPLACEMENT, MILK BASED, HIGH PROTEIN, LIQUID
Meal replacements	dry	11830800	INSTANT BREAKFAST POWDER, NOT RECONSTITUTED
Meal replacements	dry	11830810	INSTANT BFAST,PWDR,SWT W/ LO CAL SWT,NOT RECONSTUT
Meal replacements	dry	11830850	HIGH CALORIE MILK BEVERAGE, POWDER, NOT RECONST
Meal replacements	dry	11830900	PROTEIN SUPPLEMENT, MILK BASED, DRY POWDER
Meal replacements	dry	11830940	MEAL REPLACEMENT,PROTEIN,MILK BASED,FRUIT JUICE MIX
Meal replacements	dry	11830970	MEAL REPLACEMENT, PROTEIN TYPE, MILK-BASE, POWDER
Meal replacements	dry	11830990	NUTRIENT SUPP, MILK-BASE, POWDER (INCL SUSTAGEN)
Meal replacements	dry	11831500	NUTRIENT SUPPLEMENT,MILK-BASE,HIGH PROT,NOT RECONST
Meal replacements	dry	11832000	MEAL REPLACEMENT,MILK-&SOY-BASE,POWDER,NOT RECONST
Meal replacements	dry	11835100	MEAL REPLACEMENT, POSITRIM DRINK MIX, DRY POWDER
Meal replacements	dry	11835200	LOSE-IT (NANCI), MEAL REPLACEMENT, POWDER
Meal replacements	dry	41430000	PROTEIN POWDER, NFS
Meal replacements	dry	41430010	PROTEIN SUPPLEMENT, POWDERED
Meal replacements	fluid	41430200	MEAL REPLACE / SUPP, SOY-MILK-BASE,POWD,WATER ADDED
Meal replacements	dry	41430310	PROTEIN DIET POWDER W/ SOY & CASEIN
Meal replacements	solid	41435110	HIGH PROTEIN BAR, CANDY-LIKE, SOY & MILK BASE
Meal replacements	dry	41440000	TEXTURED VEGETABLE PROTEIN, DRY
Meal replacements	fluid	41440010	MEAL REPLACEMENT/SUPPLEMENT, LIQUID, HI PROTEIN
Meal replacements	fluid	41440020	ENSURE W/ FIBER, LIQUID
Meal replacements	fluid	41440050	ENSURE PLUS LIQUID NUTRITION
Meal replacements	fluid	41440100	MEAL REPLACEMENT,LIQUID,SOY-BASE (ISOCAL, OSMOLITE)
Meal replacements	solid	53540000	BREAKFAST BAR, NFS
Meal replacements	solid	53540100	BREAKFAST BAR, CAKE-LIKE
Meal replacements	solid	53540200	BREAKFAST BAR, CEREAL CRUST W/ FRUIT FILLING, LOWFAT
Meal replacements	solid	53540250	BREAKFAST BAR, CEREAL CRUST W/ FRUIT FILLING, FAT FR
Meal replacements	solid	53540500	BREAKFAST BAR, DATE, W/ YOGURT COATING
Meal replacements	solid	53541100	BREAKFAST BAR, DIET MEAL TYPE
Meal replacements	solid	53541200	MEAL REPLACEMENT BAR (INCL SLIM FAST BAR)
Meal replacements	solid	53542100	GRANOLA BAR W/ OATS, SUGAR, RAISINS, COCONUT
Meal replacements	solid	53542200	GRANOLA BAR, OATS, FRUIT, NUTS, LOWFAT
Meal replacements	solid	53542210	GRANOLA BAR, NONFAT
Meal replacements	solid	53543100	GRANOLA BAR W/ PEANUTS, OATS, SUGAR, WHEAT GERM
Meal replacements	solid	53544200	GRANOLA BAR, CHOCOLATE-COATED
Meal replacements	solid	53544210	GRANOLA BAR, W/ COCONUT, CHOCOLATE-COATED
Meal replacements	solid	53544220	GRANOLA BAR W/ NUTS, CHOCOLATE-COATED
Meal replacements	solid	53544250	GRANOLA BAR, COATED W/ NONCHOCOLATE COATING
Meal replacements	solid	53544300	GRANOLA BAR, HIGH FIBER, YOGURT COATING, NOT CHOC
Meal replacements	solid	53544400	GRANOLA BARS, W/ RICE CEREAL
Meal replacements	solid	53544450	POWERBAR (FORTIFIED HIGH ENERGY BAR)
Meal replacements	fluid	92553000	FRUIT-FLAVORED THIRST QUENCHER BEVERAGE, LOW CAL
Meal replacements	fluid	92560000	FRUIT-FLAVORED THIRST QUENCHER BEVERAGE
Meal replacements	dry	92900300	FRUIT-FLAV THIRST QUENCH BEV, DRY CONC (GATORADE)

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APPENDIX E:

Methods of Detection

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ENVIRON

Pages 000419 - 000421 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

PhastSystem™
Separation Technique File No.111

SDS-PAGE in homogeneous media

This file describes separation methods for SDS-PAGE with PhastGel homogeneous media. These methods can be used as they are, or they can be used as a guide to optimize your separations. To help you in this, a general strategy is given for optimizing separations by selecting different gel concentrations and running conditions.

This file gives only specific method information. Descriptions of how to program methods and run separations are given in the Owners Manual of PhastSystem.

Introduction

Since its introduction by Laemmli in 1970 (1), SDS-PAGE with a discontinuous buffer system has become the most common electrophoretic technique in use today. With a discontinuous buffer system, the proteins form sharp starting zones in the stacking gel, which serves to increase resolution in both gradient and homogeneous polyacrylamide (PAA) gels (2). The choice of using a gradient or homogeneous gel will depend on the complexity of the sample.

Gradient gels have many attributes, most notably; i) complex samples with a broad molecular weight (MW) range can be displayed on one gel; ii) the gradient sharpens proteins bands since the advancing edge of the migrating protein is retarded more than the trailing edge; and iii) an estimation of the MW of components in a complex sample can be made (3,4).

Although gradient gels offer superior resolution (band sharpness), homogeneous gels can offer better separation (distance between bands) to obtain optimal separation between proteins in a narrow MW range.Unlike gradient gels, homogeneous gels have a constant sieving effect on the proteins, that is, proteins do not reach a "pore-limit" that would significantly slow (or almost stop) their migration. This fact can be exploited to separate proteins in a narrow MW range from a complex sample.

A logical approach to analyzing your sample would be to first explore the complexity of the sample by electrophoresis on a gradient gel. An estimate of the MW of the components can be made. The resolution of the components may be sufficient and require no further analysis. Conversely, if the components of interest lie in a narrow MW range you may want to spread the components using a homogeneous gel. The separation can be further optimized by varying the separation time (Vh).

With PhastSystem this approach to electrophoresis is fast and easy. PhastGel gradient media can be used in conjunction with PhastGel homogeneous media and PhastSystem instrumentation to optimize separations.

In this file you will find technical data about PhastGel homogeneous media followed by suggestions for choosing the most appropriate gel for your application. Methods for running SDS-PAGE are described and listed in tables.

PhastGel homogeneous media

PhastGel homogeneous media comprise three homogeneous polyacrylamide gels: PhastGel Homogeneous 7.5, 12.5, and 20. These gels have a 13 mm stacking gel zone and a 32 mm separation gel zone. The concentration of PAA in the stacking and separation gel zones of the three media is given in table 1.

Table 1: Gel concentration in the stacking and separation gel zones of PhastGel homogeneous media.

PhastGel Media	Stacking gel zone	Separation gel zone
Homogeneous 7.5	T5%, C3%	T7.5%, C2%
Homogeneous 12.5	T6%, C3%	T12.5%, C2%
Homogeneous 20	T7.5%, C3%	T20%, C2%

The gels are approximately 0.45 mm thick and the buffer system in the gels is of 0.112 M acetate, 0.112 M Tris, pH 6.5. For SDS-PAGE, the gels are run with PhastGel SDS Buffer Strips which contain 0.2 M tricine, 0.2 M Tris, 0.55% SDS, pH 8.1.The buffer strips are made of 2% Agarose IEF.

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Separation variables

To optimize your separation in PhastGel homogeneous media, you have three variables to work with; 1) gel concentration, with three concentrations to choose from, 2) separation time, which you can vary to move the separation range in any of the three gels and 3) running conditions; depending on your sample you may, for example, choose to run a continuous rather than a discontinuous buffer separation.

Choice of gel concentration

The gel most appropriate for your sample will depend on the MW range of the components of interest. This can be determined on PhastGel Gradient 8–25 (4). You can then choose the gel with the highest selectivity for your separation as shown in figure 2 (described below).

Traditionally, gels for determining molecular weights by SDS-electrophoresis may be calibrated using a plot of log MW *versus* R_f. Such a plot produces a sigmoid curve that, between $R_f = 0.25$ and $R_f = 0.75$, approximates a straight line, which may be considered characteristic of the particular gel. A more accurate, though more complex, method uses a non-linear approach. Rodbard (5,6) has demonstrated that equation (i)

$$R_f = \frac{a}{1+(MW/c)^b} \qquad \text{(i)}$$

where a, b and c are constants*, gives a closer representation of data obtained by SDS-electrophoresis on homogeneous gels. For PhastGel™ homogeneous media, we have found that equation (ii)

$$R_f = \frac{a}{d+(MW/c)^b} \qquad \text{(ii)}$$

a modified version of the model equation (i), where a correction factor (d) has been introduced, gives the best fit to experimental results (Fig.1), even when the bromophenol blue front has been run onto the anode (although, in the latter case, the shape of the curve is altered).

From (ii), we can conveniently define the selectivity of a gel as the derivative

$-dR_f/d(\log MW)$

the slope of a tangent to the curve at a specific point (i.e., the selectivity varies with MW).

By plotting $-dRf/d(\log MW)$ *versus* log MW for each PhastGel™ homogeneous gel, we obtain the bell-shaped selectivity curves in figure 2. Note

* a is the mobility of a molecular species with MW = 0, b is a factor determining the slope of the curve, and c is the MW of a species with $R_f = a/2$. For a complete description of this mathematical model, see (5).

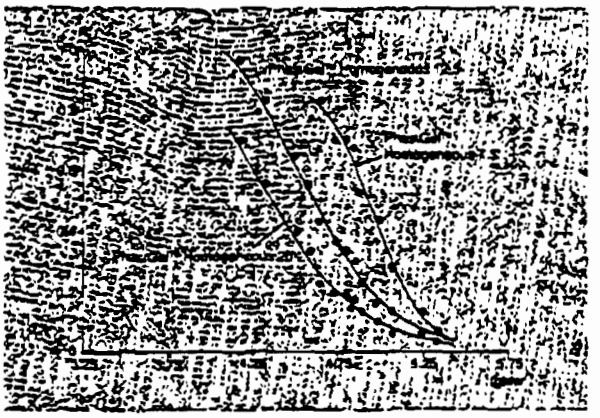

Figure 1: A plot of Rf *versus* log MW for proteins separated by SDS-PAGE using PhastGel™ homogeneous media. A modified model equation, $Rf = a/[d+(MW/c)^b]$ gives the best fit to the experimental results. Work by Larsson, A. and Edström, A. of Pharmacia LKB.

that the point of inflection on each curve occurs where MW=c. The curves in figure 2 may be used to help you choose which PhastGel homogeneous gel to use for a particular separation. For example, when you wish to separate molecules of similar molecular weight, compare the curves and choose the gel that shows the highest value of $-dRf/d(\log MW)$ at the desired MW interval.

Figure 2: Selectivity curves for PhastGel™ homogeneous media. Work by Larsson, A., Hagberg, K. and Edström, A. of Pharmacia LKB.

Choice of separation time

As homogeneous gels are made of polyacrylamide of uniform concentration throughout the separation gel zone, components that enter this zone will continue to migrate until the separation is stopped. By varying the separation time, measured in volthours (Vh), you can adjust the separation range in the gel.

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If you run your sample until the bromophenol blue band just reaches the anodic buffer strip, all the components in your sample will appear on the gel. However, your components of interest

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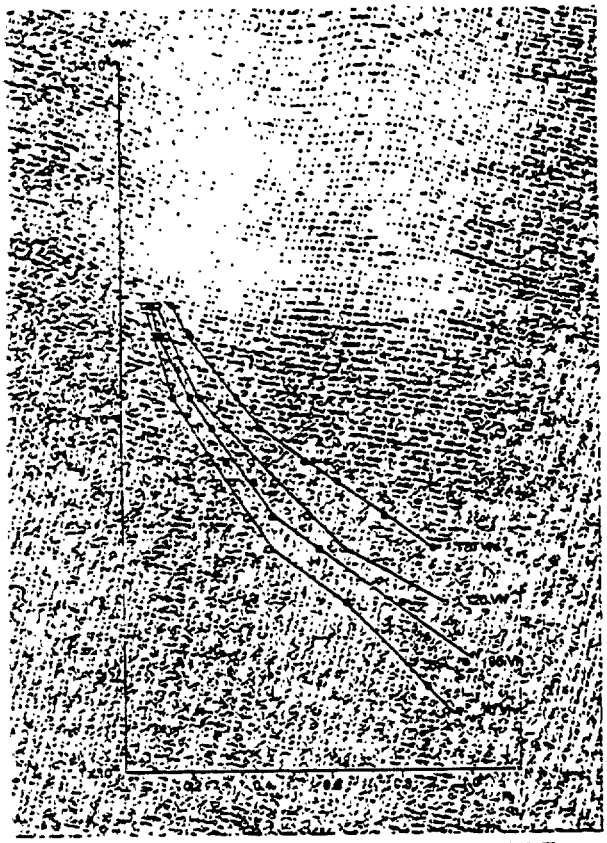

Figure 3: The shift in the separation range of PhastGel Homogeneous 7.5 with increasing Vh. Samples: SDS denatured HMW and LMW Calibration Kit proteins (Pharmacia)

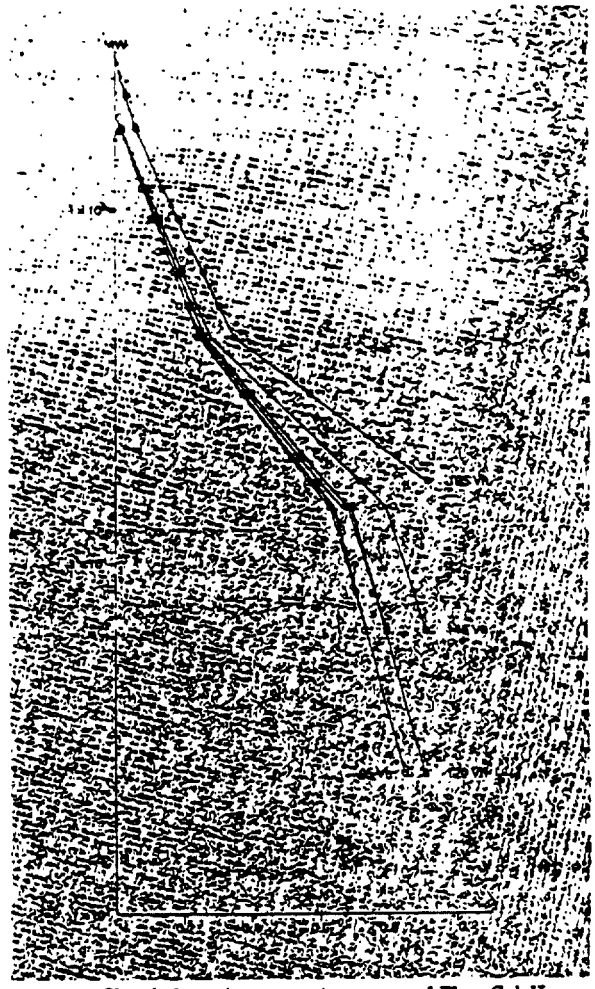

Figure 5: The shift in the separation range of PhastGel Homogeneous 20 with increasing Vh. Samples: SDS denatured HMW, LMW, and PMW Calibration Kit proteins (Pharmacia).

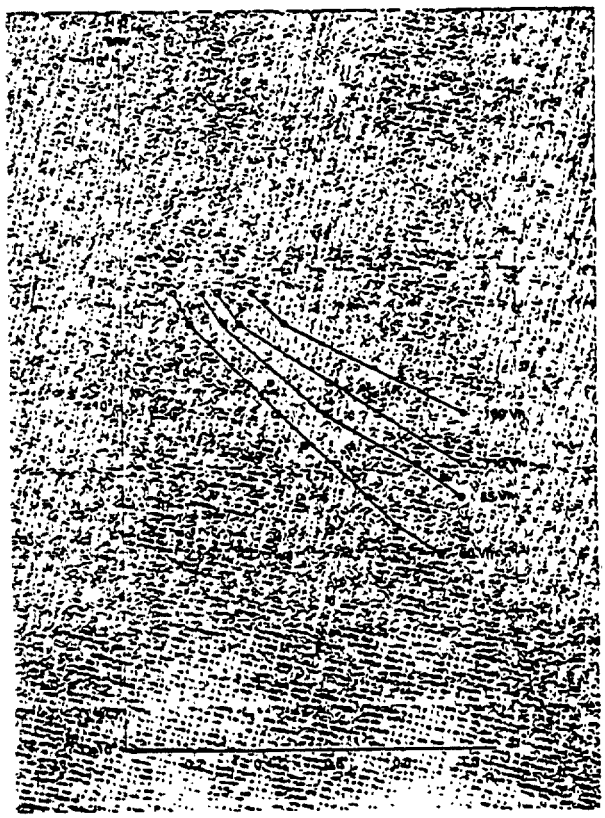

Figure 4: The shift in the separation range of PhastGel Homogeneous 12.5 with increasing Vh. Samples: SDS denatured HMW, LMW, and PMW Calibration Kit proteins (Pharmacia).

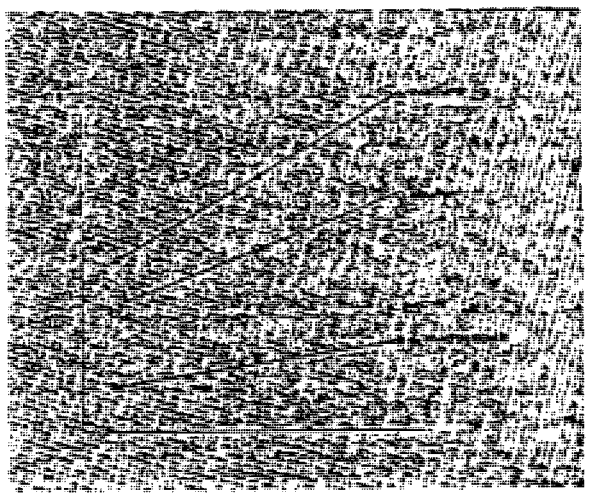

Figure 6: Migration distance of three SDS denatured proteins versus separation time (Vh). PhastGel Homogeneous 12.5.

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may not be separated to your satisfaction at that time. If the components of interest have not migrated too far in the gel, the separation may be improved by increasing the separation time. The relationship between MW and migration distance (Rf) will shift, that is, the separation range moves to a higher MW range.

On the other hand, if the components are very near the anodic buffer strip, a better separation could be obtained in a gel of higher concentration.

Figures 3, 4, and 5 illustrate how the separation ranges shift with increasing Vh using PhastGel Homogeneous 7.5, 12.5, and 20 to separate Pharmacia MW calibration proteins. Figure 6 shows the increased separation of three proteins with increasing Vh.

Choice of running conditions

Methods for running SDS-PAGE separations with a discontinuous buffer system are given in tables 3 to 5. Depending on your sample, you may choose to change these parameters. For example, if your sample components are small peptides, you may want to employ a continuous buffer system. This is done by simply applying the sample after an extended pre-run. This will prevent the smaller peptides from running with the buffer front and thereby improve the separation.

Separation ranges

Table 2 below shows the separation range for the three gels. These values are based on the time required for bromophenol blue to just reach the anodic buffer strip. This table only indicates the MW of the components that can be separated on the gels, that is, it does not indicate linearity between molecular weight and migration distance.

Table 2: MW ranges for SDS denatured proteins in PhastGel homogeneous media.

PhastGel Media	Mw-range kD	Separation time in Vh
Homogeneous 7.5	30–300	60
Homogeneous 12.5	15–250	70
Homogeneous 20	2–150	95

Estimating MW

Estimating the MW of your SDS denatured proteins in homogeneous media can be done by running calibration proteins that sufficiently cover the MW range of the components under investigation. See chapter Preservation and Evaluation in the Owners Manual of PhastSystem for a discussion on MW estimation.

The calibration curve gives a linear relationship between log MW and Rf over a major part of the MW area covered by the gel. For accuracy, estimations of MW should be made in this linear part of the calibration curve (2). As shown in figures 3, 4, and 5, this relationship occurs over certain ranges of the gel depending on the duration of the run.

Methods

In this section you will find guidelines for optimizing your separations in PhastGel homogeneous media. Cross references to relevant information in the Owners Manual of PhastSystem are given. The development methods for SDS-PAGE in Development Technique File Nos. 200 and 210 are valid for homogeneous media except for a few changes which are listed at the end of this section.

Sample preparation

Samples are prepared for SDS-PAGE as described in Separation Technique File No. 110. Note: sample overloading will distort the bands. We suggest running a dilution series when in doubt about the sample concentration.

Separation methods

Tables 3, 4, and 5 give the running conditions for PhastGel Homogeneous 7.5, 12.5, and 20 using PhastGel SDS Buffer Strips for discontinuous buffer separations.

Table 3: Separation method for SDS-PAGE with PhastGel Homogeneous 7.5. Given as method 1.

SAMPLE APPL. DOWN AT				1.2	0 Vh
SAMPLE APPL. UP AT				1.3	0 Vh
SEP 1.1	250 V	10.0 mA	3.0 W	15°C	1 Vh*
SEP 1.2	250 V	1.0 mA	3.0 W	15°C	1 Vh
SEP 1.3	250 V	10.0 mA	3.0 W	15°C	60 Vh

Table 4: Separation method for SDS-PAGE with PhastGel Homogeneous 12.5. Given as method 2.

SAMPLE APPL. DOWN AT				2.2	0 Vh
SAMPLE APPL. UP AT				2.3	0 Vh
SEP 2.1	250 V	10.0 mA	3.0 W	15°C	1 Vh*
SEP 2.2	250 V	1.0 mA	3.0 W	15°C	1 Vh
SEP 2.3	250 V	10.0 mA	3.0 W	15°C	70 Vh

Table 5: Separation method for SDS-PAGE with PhastGel Homogeneous 20. Given as method 3.

SAMPLE APPL. DOWN AT				3.2	0 Vh
SAMPLE APPL. UP AT				3.3	0 Vh
SEP 3.1	250 V	10.0 mA	3.0 W	15°C	1 Vh*
SEP 3.2	250 V	1.0 mA	3.0 W	15°C	1 Vh
SEP 3.3	250 V	10.0 mA	3.0 W	15°C	95 Vh

* For continuous buffer separations, pre-run for 60 Vh in step 1.

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The duration of the separations is specified as the volthours required for bromophenol blue to just reach the anodic buffer strip (table 2). Ensure that the bromophenol blue actually reaches the anodic buffer strip before stopping the separation.

The methods contain 3 steps: step 1 is to allow the buffer front to reach the point of sample application (for discontinuous buffer system); step 2 is for sample application at reduced current (to prevent streaking); and step 3 for driving the components through the gel where they separate according to their size.

Figures 7, 8, and 9 show examples of results using PhastGel homogeneous media with the methods described above.

Note: These running conditions are to serve as guidelines. For your sample, other conditions could be optimal.

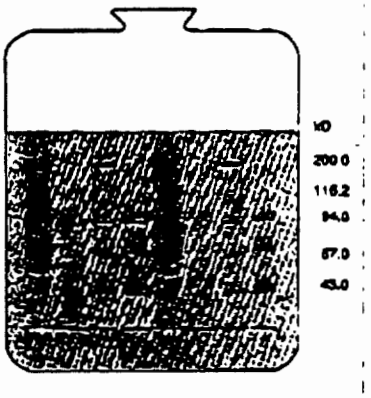

Figure 7: An example of SDS-PAGE with PhastGel Homogeneous 7.5 and coomassie staining. The samples from left to right: human plasma, *E.coli* extract, HMW and LMW calibration proteins. Sample to result: 60 min.

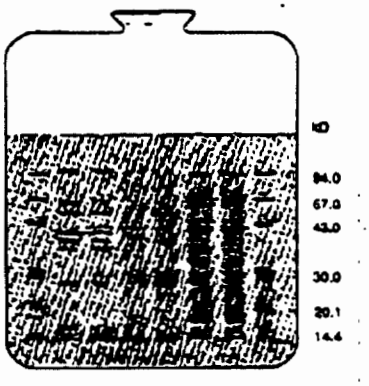

Figure 8: An example of SDS-PAGE with PhastGel Homogeneous 12.5 and coomassie staining. The samples from left to right: LMW calibration proteins, fish muscle extract, *E. coli* extract, and bovine muscle extract. Sample to result: 60 min.

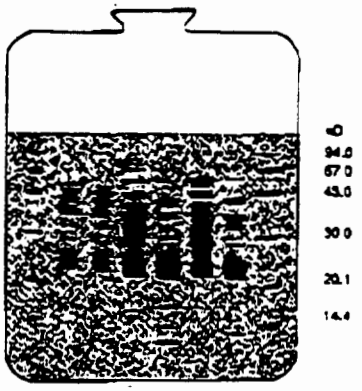

Figure 9: Example of SDS-PAGE with PhastGel Homogeneous 20 and silver staining. Samples are from early steps in the purification of rH-growth hormones (kindly donated by Kabi Vitrum). Sample to result: 95 min.

Coomassie staining

Table 2 in Development Technique File No. 200 describes a method for coomassie staining PhastGel™ gradient media This method is valid for PhastGel homogeneous media with the following changes in glycerol concentration in the preserving step.(step 5 in table 2):

Homogeneous 7.5: Use 2.5% glycerol
Homogeneous 12.5: Use 5% glycerol
Homogeneous 20: Use 10% glycerol

The concentration of the acetic acid is the same for the three gels, *i.e.* 10%.

Silver staining

Table 2 in Development Technique File No. 210 describes a method for silver staining PhastGel gradient media. This method is also valid for PhastGel homogeneous media with the following changes in glycerol concentration in the preserving step (step 15 in table 2):

Homogeneous 7.5: Use 2.5% glycerol
Homogeneous 12.5: Use 5% glycerol.
Homogeneous 20: Use 10% glycerol.

The concentration of acetic acid is the same for all three gels *i.e.* 10%.

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Pharmacia

Pharmacia LKB Biotechnology
S-751 82 Uppsala, Sweden

PhastSystem™ Development Technique File No. 200

Fast coomassie staining

This technique file describes optimized methods for detecting proteins in PhastGel™ IEF and gradient media by coomassie staining. The methods are designed to give reproducible, fast results with good sensitivity and low background staining. Results are obtained within 25–45 minutes, depending on gel type and sensitivity required for the application.

The average sensitivity limit of this technique is estimated to be approximately 20 to 30 ng of protein per band. This is based on serial dilution studies of the Pharmacia Calibration Kit proteins.

Introduction

The coomassie staining technique presented in this technique file has been optimized for detecting proteins in PhastGel separation media using PhastGel Blue R. PhastGel Blue R is a coomassie R 350 dye in readily soluble tablet form.

Coomassie, a triphenylmethane anionic dye, preferentially forms dye complexes with proteins in the gel matrix. The rate of complex formation is limited essentially by the rate of diffusion of the dye into the gel.

Since PhastGel IEF and gradient media are approximately 0.35 mm and 0.45 mm thick respectively, reagents will diffuse quickly into the gels. To increase the diffusion rate, the gels are spun in the development solutions, and the reactions are performed at elevated temperatures. Thin gels and accelerated diffusion gives fast protein detection. Results are reproducible since the duration and the operating temperature of each step follows the programmed method.

Once the proteins are sufficiently stained, excess coomassie is washed from the gel matrix leaving the dark blue bands against a clear background. This destaining step is essentially limited by the rate of diffusion of the coomassie molecules from the gel matrix into the surrounding solution. For a more detailed discussion of this mechanism, we refer you to (1), (2), and (3).

After development, the gels are dried. Dried gels are easily stored in notebooks, or cut and mounted in slide frames.

Materials and Methods

PhastGel IEF media

Prepare the following solutions for PhastGel IEF media. The solutions are listed in order of use. A volume of 80 ml is required to fill the chamber and tubing (for every step).

Label all bottles with their corresponding in-port number according to the programmed method.

1. Fix: 20% trichloroacetic acid. This solution can be recycled 3 to 4 times.
2. Wash/Destain: 30% methanol and 10% acetic acid in distilled water (3:1:6). (This solution can be recycled 3 to 4 times.)
3. Stain: 0.02% PhastGel Blue R solution in approximately 30% methanol and 10% acetic acid in distilled water and 0.1% (w/v) $CuSO_4$.

   Stock solution: dissolve 1 PhastGel Blue R tablet in 80 ml of distilled water by stirring for 5–10 minutes. Add 120 ml of methanol and stir for 2 minutes. This makes a 0.2% solution.

   Final solution: mix 1 part of filtered stock solution with 9 parts of a methanol:acetic acid:distilled water (3:1:6) solution listed in step 2 above. Add $CuSO_4$ to 0.1% (w/v). The $CuSO_4$ is added to decrease the background staining (4).

   Make the final solution fresh the day you plan to use it. Do not recycle this solution.

Program the method in table 1 into the development method file for coomassie staining of PhastGel IEF 3–9, 5–8, and 4–6.5. This method is valid for electrophoretic titration curve analysis using these media.

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Table 1: Coomassie staining method for PhastGel IEF media to program into the development method file.

Step number	Solution	IN-port	OUT-port	Time	Temperature
1	Fix	1	1	5 min	20°C
2	Wash/Destain	2	0	2 min	20°C
3	Stain	3	0	10 min	50°C
4	Wash/Destain	2	0 (or 5)	10 min	50°C

[1] The destaining solution in step 4 can be filtered through activated charcoal and recycled 3 to 4 times. Use out-port 5 to collect the solution for recycling.

PhastGel electrophoresis media

Prepare the following solutions for PhastGel electrophoresis media. The solutions are listed in order of use. A volume of 80 ml is required to fill the chamber and tubing (for every step).

Label the bottles with their corresponding in-port number according to the programmed method.

1. Stain: 0.1% PhastGel Blue R solution in 30% methanol and 10% acetic acid in distilled water.

   Stock solution: dissolve 1 tablet of PhastGel Blue R in 80 ml of distilled water and stir for 5–10 minutes. Add 120 ml of methanol and stir for 2–3 minutes. This makes a 0.2% solution.

   Final solution: mix 1 part of filtered stock solution with 1 part of 20% acetic acid in distilled water.

   Prepare this solution fresh the day you plan to use it. Do not recycle this solution.

2. Destain: 30% methanol and 10% acetic acid in distilled water (3:1:6).

   Prepare at least 300 ml of this solution; enough to fill the chamber three times.

3. Destain: use the solution prepared for step 2.

4. Destain: use the solution prepared for step 2.

5. Preserving solution: X% glycerol and 10% acetic acid in water.

   Note: The concentration of glycerol is different for different gels.
   2.5% for PhastGel Homogeneous 7.5
   5% for PhastGel 10–15 and 12.5
   10% for PhastGel 8–25 and 20.

   Do not recycle this solution. This solution will help to keep the gels flexible and resistant to cracking.

Program the method in table 2 into the development method file for SDS-PAGE. Program the method in table 3 into the development method file for native-PAGE. Native-PAGE gels take about 10 minutes longer to destain than SDS-PAGE gels.

Table 2: Coomassie staining method for SDS-PAGE with PhastGel electrophoresis media to program into the development method file.

Step number	Solution	IN-port	OUT-port	Time	Temperature
1	Stain	4	0	8 min	50°C
2	Wash/Destain	2	0 (or 5)[1]	5 min	50°C
3	Wash/Destain	2	0 (or 5)	8 min	50°C
4	Wash/Destain	2	0 (or 5)	10 min	50°C
5	Preserving solution	9	0	5 min	50°C

[1] The destaining solutions in steps 2, 3, and 4 can be filtered through activated charcoal and recycled 3 to 4 times. Use out-port 5 to collect the solutions for recycling.

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Table 3: Coomassie staining method for native-PAGE with PhastGel electrophoresis media to program into the development method file.

Step number	Solution	IN-port	OUT-port	Time	Temperature
1	Stain	4	0	7 min	50°C
2	Destain	2	0 (or 5)[1]	1 min	50°C
3	Destain	2	0 (or 5)	10 min	50°C
4	Destain	2	0 (or 5)	15 min	50°C
5	Preserving solution	3	0	15 min	50°C

[1] The destaining solutions in steps 2, 3, and 4 can be filtered through activated charcoal and recycled 3 to 4 times. Use out-port 5 to collect the solutions for recycling.

Procedure

The procedure for programming and running the development methods in tables 1, 2, and 3 is the same for both PhastGel IEF and gradient media: program the method(s), connect the solutions to the correct ports, insert the gel(s), close the lid and press the start button. Full instructions are given in the chapter on Development Procedures in the System Guide.

Check list

Before starting development methods you should run through the following checklist to avoid easily made mistakes:

1. Are the bottles connected correctly?
2. Are the tubes securely submerged in the solutions? (Also, check for kinks or obstructions in the tubing).
3. If 2 gels are developed simultaneously: are the gels inserted so that their surfaces face one another?
4. If 1 gel is developed: is the gel in the lower position of the gel holder, and is the gel surface facing upwards?
5. Is the lid closed tightly?
6. Have you chosen the correct method from the development file?

Sensitivity

The sensitivity of this technique was estimated using serial dilutions of the Pharmacia Calibration Kit proteins (for isoelectric point, pI, and molecular weight, MW, measurements). Examples are shown in figures 1 and 2 below.

The sensitivity will depend on the protein, since different proteins bind different amounts of coomassie. The sensitivity limit of this technique is expressed as an average for the proteins studied. The limit is 20 to 30 ng protein/band using the Sample Applicator 8/1 (8 lanes, each 1 µl). The bands are approximately 3 mm wide with this applicator.

Figure 1: Serial dilution of the Pharmacia Broad Range pI Calibration Kit on PhastGel IEF 3–9. Each vial in the kit contains approximately 20 µg of each of the proteins listed below. The dilution volume/vial is, starting from the left: 50 µl, 75 µl, 100 µl, 200 µl, 400 µl, 600 µl, 800 µl, and 1 ml. The samples were applied in 1 µl volumes to each of 8 lanes.

From cathode to anode, the proteins are:
lentil lectin (basic)
lentil lectin (middle)
lentil lectin (acidic)
myoglobin (basic)
myoglobin (acidic)
human carbonic anhydrase B
bovine carbonic anhydrase B
β-lactoglobulin A
soybean trypsin inhibitor
amyloglucosidase

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Figure 2: Serial dilution of the Pharmacia LMW Calibration Kit (denatured) on PhastGel Gradient 10–15 with SDS buffer strips. Each vial contains approximately 100 µg of each of the proteins listed below. The dilution volume/vial is, starting from the left: 200 µl, 400 µl, 800 µl, 1.6 ml, 3.2 ml, 6.4 ml, and 12.8 ml. The samples were applied in 1 µl volumes to each of 7 lanes (the first lane is empty).

From cathode to anode, the proteins are:
phosphorylase b
albumin
ovalbumin
carbonic anhydrase
trypsin inhibitor
α-lactalbumin

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Pharmacia

Pharmacia LKB Biotechnology
S-751 82 Uppsala, Sweden

Pages 000432 - 000439 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Submission End

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ENVIRON

June 27, 2000

Dr. Linda Kahl
Office of Premarket Approval, HFS-200
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, SW
Washington, DC 20204

RE: GRAS Notice (GRN) No. 000042

Dear Dr. Kahl:

In response to your question during our phone conversation regarding GRAS Notice (GRN) No. 000042 from DMV International, I would like to clarify the use of bovine lactoferrin in infant formula. The infant formula for which the product is intended as an ingredient is restricted to cow milk based infant formula.

I hope this clarification is of help. Please contact me if any further questions arise.

Thank you,

Claire L. Kruger, Ph.D., D.A.B.T.
Senior Science Manager

2000 JUN 29 P 2:50

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4350 North Fairfax Drive • Suite 300 • Arlington, Virginia 22203 • USA • Tel: (703) 516-2300 • Fax: (703) 516-2345
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September 11, 2000

Dr. Paulette Gaynor
Office of Premarket Approval, HFS-200
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, SW
Washington, DC 20204

Dear Dr. Gaynor:

We wish to notify you that DMV International requests that FDA stop evaluation of bovine lactoferrin as a "generally recognized as safe" ("GRAS") ingredient for use in infant formulas. DMV International requests that FDA continue the ongoing GRAS evaluation of bovine lactoferrin for use as an ingredient in sports and functional foods, as described in GRN No. 000042.

DMV International will, at a future time, resubmit a Notification for the determination of bovine lactoferrin as a GRAS ingredient for use in infant formulas. If you have any questions concerning this matter, please feel free to contact Claire Kruger, Ph.D., Senior Science Manager, ENVIRON Corporation, 4350 North Fairfax Drive, Suite 300, Arlington, VA. 22203, telephone: (703) 516-2309, facsimile: (703) 516-2393.

Sincerely,

Claire L. Kruger, Ph.D., D.A.B.T.
Senior Science Manager

cc: V. Frankos
R. Nimmagudda

2000 SEP 13 A 7:45

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January 12, 2001

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OFFICE OF
PREMARKET APPROVAL

Dr. Paulette Gaynor
Office of Premarket Approval, HFS-200
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, SW
Washington, DC 20204

Dear Dr. Gaynor:

We wish to notify you that DMV International is submitting the attached addendum to the GRAS Notification for bovine lactoferrin for use as an ingredient in sports and functional foods, as described in GRN No. 000042.

The addendum contains a revised calculation of the background exposure to lactoferrin through dairy products and whey products added to food. In addition, a revised calculation of the estimated daily intake (EDI) is included. Both the revised calculation of background exposure and the revised EDI should replace the original calculations presented in GRN No. 000042.

The background intake of lactoferrin was completed by estimating per capita lactoferrin intake from milk proteins in dairy products and adding this to the estimated per capita lactoferrin intake from whey products. These estimates of intake of lactoferrin from both dairy sources and whey protein added to food indicate that background exposure can be as high as 100 mg lactoferrin per person per day.

This estimate of lactoferrin exposure is supported by calculations of potential lactoferrin intakes based on current recommends in the U.S. for consumption of milk and milk products. In its Food Guide Pyramid, the United States Department of Agriculture recommends daily intake of 2 to 3 servings of milk products (USDA 1996). Individuals consuming 3 cups of milk per day, or 3 servings, would have lactoferrin intakes of approximately 71 mg per day. Added to lactoferrin intake from whey protein sources, total lactoferrin intake is again approximately 100 mg per day.

In GRN No. 000042, the EDI for bovine lactoferrin was proposed to be 1.0 and 2.1 g/person/day at the mean and 90th percentile of consumption. A revised EDI for bovine lactoferrin has been calculated for its proposed use as an ingredient in functional foods in the form of ready-to-drink beverages, beverage powder mixes, and bars. The proposed use level of lactoferrin is 100 mg per product serving. The proposed EDI of lactoferrin from these uses is estimated by product category and from all categories combined. These estimates were based on reported consumption of foods representative of the proposed use categories that were reported in the 1994-96 Continuing Survey of Food Intakes by Individuals (CSFII) and its 1998 Supplemental Children's Survey by

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individuals ages 2 years and older (USDA, 2000). The EDI from all use categories combined is 105 mg per day, which is equivalent to approximately one serving of the proposed foods per day. The 90th percentile of intake for all categories combined is 196 mg, or approximately two servings of the proposed products per day. It is important to note that nearly all consumers included in these estimates reported intake of a product from just one use category.

The information provided in GRN 000042 summarizes the basis for the GRAS determination of bovine lactoferrin, based on scientific procedures. An extensive database, consisting of both animal and human exposure and safety data, is available for determination of the safety of bovine lactoferrin for use an ingredient in sports and functional foods. No evidence of adverse health effects have been determined in animal studies at up to 2 g/kg/day or in human clinical trials at up to 3.6 g/day. A revised estimate of background intake indicates that background exposure can be as high as 100 mg lactoferrin/person/day.

The proposed EDI of 105 mg/person/day (mean intake) will result in no more than a doubling of potential background exposure and is far less than levels of 3.6 g/day shown to be safe in humans and 2 g/kg/day shown to be safe in animals. There is no evidence in the available information on bovine lactoferrin that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when it is used at the levels that are now current or might reasonably be expected from the proposed applications. Based on proposed use levels, DMV International concludes that bovine lactoferrin is GRAS for use as an ingredient in sports and functional foods.

Sincerely,

Claire L. Kruger, Ph.D., D.A.B.T.
Senior Science Manager

cc: Bill Frankos
Ram Nimmagudda

Attachments

000451

Background Exposure to Bovine Lactoferrin from Dairy Products and Whey Products Added to Food

Bovine lactoferrin has been consumed by humans for thousands of years as a naturally occurring protein found in milk. Current sources of lactoferrin in the American diet include milk proteins naturally occurring in milk and milk products, and whey proteins added to foods throughout the food supply in the form of whey products. Milk proteins and whey proteins contain approximately 0.03 g and 1.4 g lactoferrin per 100 g, respectively (Barth and Behnke 1997).

Using food intake data reported in the United States Department of Agriculture's (USDA) 1994-96 Continuing Survey of Food Intakes by Individuals (CSFII) and its 1998 Supplemental Children's Survey (USDA 2000), ENVIRON determined the average per capita intake of milk proteins from milk and milk products (Table 1).

The CSFII 1994-96 was conducted between January 1994 and January 1997 with non-institutionalized individuals in the United States. In each of the three survey years, data were collected from a nationally representative sample of individuals of all ages. The CSFII 1998 was a survey of children ages 0 through 9 which was supplemental to the CSFII 1994-96. It used the same sample design as the CSFII 1994-96 and was intended to be merged with CSFII 1994-96 to increase the sample size for children. The merged surveys are designated as CSFII 1994-96, 1998. In the CSFII 1994-96, 1998, dietary intakes were collected through in-person interviews using 24-hour recalls on two nonconsecutive days approximately one week apart. A total of 21,662 individuals provided data for the first day; of those individuals, 20,607 provided data for a second day. The survey database includes data on 52 dietary components, including protein, for each food reported.

The estimates of milk protein intake were based on reported consumption of all milk and milk products including fluid and flavored milk, dry and reconstituted milk, milk-based beverage mixes, milk and milk products in beverage mixtures, yogurt, ice cream, cream, sour cream, and puddings. The analyses did not include cheese, infant formulas, meal replacements, whey-based beverages and beverage mixes, or butter. Additionally, the milk and milk products that were ingredients in food mixtures coded as a single item (i.e., milk used in the preparation of canned soup) were not included in the estimates. Given that whey proteins may be added to some milk products such as yogurt, ice cream, and beverage mixes, we assumed only 75 percent of the protein content of milk products to be milk protein; this allows for the addition of approximately one gram of whey protein per 100 grams of each milk product. The food codes included in the estimates of milk protein intake are listed in Appendix A.

The mean intake of milk proteins by individuals ages 2 years and older in the U.S. is approximately 7.7 g per day. This is equivalent to approximately 23 mg lactoferrin per day. The 90th percentile of milk protein intake in the U.S. is approximately 18.6 g per day, or 56 mg lactoferrin per day. The 95th percentile of milk protein intake is 23.5 g per

day, or approximately 70 mg lactoferrin. It is important to note that these estimates do not reflect the lactoferrin contained in milk and milk products used as an ingredient and a considerable allowance was made for the potential presence of whey proteins in milk products. Therefore, these estimates may tend to underestimate current consumption of milk proteins and consequently lactoferrin from milk proteins.

Estimates of whey protein intake were derived from estimates of whey products generated by FDA (Rulis 2000). FDA estimated that the current consumption of whey products on a per capita basis is approximately 4.0 grams per person per day. ENVIRON has assumed that the FDA estimate of "whey products" represents the usual intake of whey from all uses in the U.S. food supply. Whey and whey products may be used in food in accordance with good manufacturing practice and are used in foods for a wide variety of functional uses. Some of the uses of whey and whey products include: nutrient, solvent/carrier/encapsulating agent, material handling aid, appearance control agent for substances other than colors and color modifiers, leavening agent, stabilizer and thickener, flavor ingredient of flavor modifier, color control agent, texturizer, and malting/fermenting aid (Burdock 1997).

The protein content of whey products varies depending on the specific type and concentration. From current regulatory approvals and specifications for whey and whey products (21 CFR §184.1979 a,b,c) protein concentration can range from 10 percent to not less than 25 percent of the products. Whey protein isolate (GRN000037) contains not less than 90 percent protein. Therefore, in order to calculate protein exposures from the whey product consumption estimate, a realistic estimate of 25 percent protein was used. Using this assumption, we estimate that the per capita intake of whey protein is approximately 1.0 g per day. This is equivalent to 14 mg lactoferrin per day. We estimate that the 90th percentile of whey protein intake is approximately twice the mean intake, or 2.0 g per day, which in turn is equivalent to approximately 84 mg lactoferrin per day.

Estimates of the per capita lactoferrin intake from milk proteins in dairy products were added to the estimated per capita lactoferrin intake from whey products. These estimates of intake of lactoferrin from both dairy sources and whey protein added to food indicate that background exposure can be as high as 100 mg lactoferrin per person per day.

This estimate of lactoferrin exposure is supported by calculations of potential lactoferrin intakes based on current recommends in the U.S. for consumption of milk and milk products. In its Food Guide Pyramid, the United States Department of Agriculture recommends daily intake of 2 to 3 servings of milk products (USDA 1996). Individuals consuming 3 cups of milk per day, or 3 servings, would have lactoferrin intakes of approximately 71 mg per day. Added to lactoferrin intake from whey protein sources, total lactoferrin intake is again approximately 100 mg per day.

TABLE 1
Estimate of Per Capita Daily
Intake of Bovine Lactoferrin from Milk Proteins and Whey Proteins

	Milk Proteins		Whey Proteins		Milk & Whey Proteins
	Milk Proteins in Dairy Products [a] (g)	Lactoferrin from Milk Proteins in Dairy Products [a, b] (mg)	Whey Proteins in the Food Supply [c] (g)	Lactoferrin from Whey Proteins in the Food Supply [c, d] (mg)	Lactoferrin from Milk and Whey Proteins [e] (mg)
Mean Intake	7.7	23	1.0	14	37
90th Percentile of Intake	18.6	56	2.0	28	84
95h Percentile of Intake	23.5	70	2.0 [f]	28 [f]	98

[a] Data source: USDA 1994-96, 1998 Continuing Survey of Food Intakes by Individuals; estimates reflect intake of protein from milk and milk products including fluid and flavored milk, dry and reconstituted milk, milk-based beverage mixes, yogurt, ice cream, cream, sour cream, and puddings. Estimates include all individuals 2 years and older who provided two 24-hour diet recalls. Estimates generated with USDA sampling weights and WesVar Complex Samples Version 3.0.
[b] Calculation of lactoferrin based on 0.03% lactoferrin in milk protein (Barth and Behnke 1997).
[c] Data source: FDA estimate of per capita whey products consumption (Rulis 2000; Agency Response Letter to GRAS Notice No. GRN 000037); assumed 25% whey protein in whey products; assumed 90th percentile of intake is twice the mean intake.
[d] Calculation of lactoferrin based on 1.4% lactoferrin in whey protein (Barth and Behnke 1997).
[e] Represents sum of estimates of lactoferrin from milk proteins in dairy products and lactoferrin from whey proteins in the food supply.
[f] Unable to estimate 95th percentile; values represent estimate of 90th percentile.

000454

Estimated Daily Intake (EDI) of Bovine Lactoferrin from Proposed Uses

Bovine lactoferrin is proposed for use as an ingredient in functional foods in the form of ready-to-drink beverages, beverage powder mixes, and bars. The proposed use level of lactoferrin is 100 mg per product serving. The serving sizes for these types of products as identified by the Reference Amounts Customarily Consumed (21 CFR § 101.12) are as follows:

Ready-To-Drink Beverages	240 mL or one can
Beverage mixes	amount used to prepare one 240 mL serving
Bars	40 g or one bar

The EDI of lactoferrin from the proposed uses in ready-to-drink beverages, beverage mixes (powders), and bars is presented in Table 2. The proposed EDI of lactoferrin from these uses is estimated by product category and from all categories combined. These estimates were based on reported consumption of foods representative of the proposed use categories that were reported in the 1994-96 Continuing Survey of Food Intakes by Individuals (CSFII) and its 1998 Supplemental Children's Survey by individuals ages 2 years and older (USDA, 2000). A list of the food codes used in the estimates is provided in Appendix B. Default serving sizes (in grams) for each of the food codes were identified using the USDA Survey Food Coding Database (USDA, 2000).

The EDI from all use categories combined is 105 mg per day, which is equivalent to approximately one serving of the proposed foods per day. The 90th percentile of intake for all categories combined is 196 mg, or approximately two servings of the proposed products per day. It is important to note that nearly all consumers included in these estimates reported intake of a product from just one use category.

TABLE 2
Estimates of Daily Intake of Bovine Lactoferrin from Proposed Uses in Functional Foods at 100 mg per Serving [a]: Ready-To-Drink Beverages, Beverage Mixes and Bars

Use Category	Users		Intake per Day per User	
	Number of People	Percent of the Survey Population	Mean Intake (mg)	90th Percentile Intake (mg)
Ready-to-drink Beverages	110	0.8	105	188
Beverage mixes	146	1.1	110	195
Bars	52	0.4	67	112
All proposed uses	299	2.2	105	196

[a] Data source: USDA 1994-96, 1998 Continuing Survey of Food Intakes by Individuals. Estimates based on 2-day average intakes reported by users (ages 2 years and older) of the food codes as identified in Appendix B. Estimates generated with USDA sampling weights and WesVar Complex Samples Version 3.0.

000456

References:

Barth CA and Behnke U. 1997. Nutritional significance of whey and whey components. *Nahrung* 41:S2-S12.

Burdock GA. 1997. *Encyclopedia of Food and Color Additives*. Boca Raton, FL: CRC Press, Inc.

Rulis AM. 2000. *Agency Response Letter GRAS Notice No. GRN 000037*. Accessed at http://vm.cfsan.fda.gov/~rdb/opa-g037.html.

U.S. Department of Agriculture. 1996. *Food Guide Pyramid*. Washington, DC: U.S. Department of Agriculture, Center for Nutrition Policy and Promotion. Home and Garden Bulletin No. 252.

U.S. Department of Agriculture, Agricultural Research Service. 2000. *1994-96, 1998 Continuing Survey of Food Intakes by Individuals* [CD-ROM], data and documentation. National Technical Information Service, Accession No. PB2000-500027.

Appendix A: Food codes in the 1994-96, 1998 Continuing Survey of Food Intakes by Individuals used to estimate per capita intake of milk protein

Food Code	Food Name	Percent of Protein Assumed to be Naturally Occurring in Milk
11100000	MILK, NFS	100
11111000	MILK, COW'S, FLUID, WHOLE	100
11111160	MILK, CALCIUM FORTIFIED, COW'S, FLUID, 1% FAT	100
11111170	MILK, CALCIUM FORTIFIED, SKIM/NONFAT, COW, FLUID	100
11112000	MILK, COW'S, FLUID, NOT WHOLE, NS AS TO % FAT	100
11112110	MILK, COW'S, FLUID, 2% FAT	100
11112120	MILK, COW'S, FLUID, ACIDOPHILUS, 1% FAT	100
11112130	MILK, COW'S, FLUID, ACIDOPHILUS, 2% FAT	100
11112210	MILK, COW'S, FLUID, 1% FAT	100
11113000	MILK, COW'S, FLUID, SKIM OR NONFAT	100
11114300	MILK, LOW LACTOSE, 1% FAT	100
11114310	MILK, LOW LACTOSE, 1% FAT, FORTIFIED WITH CALCIUM	100
11114320	MILK, LOW LACTOSE, NONFAT	100
11114321	MILK, LOW LACTOSE, NONFAT, W/ CALCIUM	100
11114330	MILK, COW'S FL LACTOSE REDUCED 2% FAT (LACTAID)	100
11115000	BUTTERMILK, FLUID (INCLUDE KEFIR MILK)	100
11115200	BUTTERMILK, FLUID, 2% FAT	100
11116000	MILK, GOAT'S, FLUID, WHOLE	100
11120000	MILK, DRY, RECONSTITUTED, NFS	100
11121100	MILK, DRY, RECONSTITUTED, WHOLE	100
11121210	MILK, DRY, RECONSTITUTED, LOWFAT	100
11121300	MILK, DRY, RECONSTITUTED, NONFAT	100
11210000	MILK, EVAPORATED, NS AS TO FAT CONTENT & DILUTION	100
11210050	MILK, EVAPORATED, NS FAT & DILUTION, IN COFFEE/TEA	100
11210100	MILK, EVAPORATED, NS AS TO FAT, UNDILUTED	100
11210200	MILK, EVAPORATED, NS AS TO FAT, DILUTED	100
11211000	MILK, EVAPORATED, WHOLE, NS AS TO DILUTION	100
11211050	MILK, EVAPORATED, WHOLE, IN COFFEE/TEA	100
11211100	MILK, EVAPORATED, WHOLE, UNDILUTED	100
11211200	MILK, EVAPORATED, WHOLE, DILUTED	100
11211400	MILK, EVAPORATED, 2% FAT, NS AS TO DILUTION	100
11211500	MILK, EVAPORATED, 2% FAT, UNDILUTED	100
11212000	MILK, EVAPORATED, SKIM, NS AS TO DILUTION	100
11212050	MILK, EVAPORATED, SKIM, IN COFFEE/TEA	100
11212100	MILK, EVAPORATED, SKIM, UNDILUTED	100
11220000	MILK, CONDENSED, SWEETENED, NS AS TO DILUTION	100
11220100	MILK, CONDENSED, SWEETENED, UNDILUTED	100
11220200	MILK, CONDENSED, SWEETENED, DILUTED	100
11410000	YOGURT, NS AS TO TYPE OF MILK/FLAVOR	75

11411010	YOGURT, PLAIN, NS AS TO TYPE OF MILK	75
11411100	YOGURT, PLAIN, WHOLE MILK	75
11411200	YOGURT, PLAIN, LOWFAT MILK	75
11411300	YOGURT, PLAIN, NONFAT MILK	75
11420000	YOGURT, VANILLA, LEMON, COFFEE, NS AS TO MILK TYPE	75
11421000	YOGURT, VANILLA, LEMON, COFFEE, WHOLE MILK	75
11422000	YOGURT, VANILLA, LEMON, COFFEE, LOWFAT MILK	75
11423000	YOGURT, VANILLA, LEMON, COFFEE, NONFAT MILK	75
11424000	YOGURT, VANILLA, LEMON, COFFEE, NONFAT MILK, LOW CAL	75
11425000	YOGURT, CHOCOLATE, NS AS TO TYPE OF MILK	75
11426000	YOGURT, CHOCOLATE, WHOLE MILK	75
11427000	YOGURT, CHOCOLATE, NONFAT MILK	75
11430000	YOGURT, FRUIT VARIETY, NS AS TO MILK TYPE	75
11431000	YOGURT, FRUIT VARIETY, WHOLE MILK	75
11432000	YOGURT, FRUIT VARIETY, LOWFAT MILK	75
11433000	YOGURT, FRUIT VARIETY, NONFAT MILK	75
11433500	YOGURT, FRUITED, NONFAT MILK, LOW CAL SWEETENER	75
11459990	YOGURT, FROZEN, NS AS TO FLAVOR, NS TO TYPE OF MILK	75
11460000	YOGURT, FROZEN, NOT CHOCOLATE, TYPE OF MILK NS	75
11460100	YOGURT, FROZEN, CHOCOLATE, TYPE OF MILK NS	75
11460150	YOGURT, FROZEN, NS AS TO FLAVOR, LOWFAT MILK	75
11460160	YOGURT, FROZEN, CHOCOLATE, LOWFAT MILK	75
11460170	YOGURT, FROZEN, NOT CHOCOLATE, LOWFAT MILK	75
11460190	YOGURT, FROZEN, NS AS TO FLAVOR, NONFAT MILK	75
11460200	YOGURT, FROZEN, CHOCOLATE, NONFAT MILK	75
11460250	YOGURT,FROZEN,NOT CHOCOLATE,W/ SORBET/SORBET-	75
11460300	YOGURT, FROZEN, NOT CHOCOLATE, NONFAT MILK	75
11460400	YOGURT,FRZ,CHOCOLATE,NONFAT MILK,W/ LOW-CAL SWEET	75
11460410	YOGURT,FRZ,NOT CHOC,NONFAT MILK,W/ LOW-CAL SWEET	75
11460420	YOGURT, FROZEN, NS AS TO FLAVOR, WHOLE MILK	75
11460430	YOGURT, FROZEN, CHOCOLATE, WHOLE MILK	75
11460440	YOGURT, FROZEN, NOT CHOCOLATE, WHOLE MILK	75
11461200	YOGURT, FROZEN, SANDWICH	75
11461250	YOGURT, FROZEN, CONE, CHOCOLATE	75
11461260	YOGURT, FROZEN, CONE, NOT CHOCOLATE	75
11461270	YOGURT, FROZEN, CONE, NOT CHOCOLATE, LOWFAT MILK	75
11511000	MILK, CHOCOLATE, NFS	75
11511100	MILK, CHOCOLATE, WHOLE MILK BASED	75
11511200	MILK, CHOCOLATE, REDUCED FAT MILK BASED	75
11511300	MILK, CHOCOLATE, SKIM MILK BASED	75
11512000	COCOA,HOT CHOCOLATE,NOT FROM DRY MIX, W/WHOLE	75
11512500	HOT CHOCOLATE DRINK W/ EVAP MILK, PUERTO RICAN	75
11513000	COCOA & SUGAR MIXTURE, MILK ADDED, NS TYPE MILK	75
11513100	COCOA & SUGAR MIXTURE, WHOLE MILK ADDED	75
11513150	COCOA & SUGAR MIX, RED FAT MILK ADDED	75
11513200	COCOA & SUGAR MIXTURE, LOWFAT MILK ADDED	75
11513300	COCOA & SUGAR MIXTURE, SKIM MILK ADDED	75

11513400	CHOCOLATE SYRUP MILK ADDED, NS AS TO TYPE OF MILK	75
11513500	CHOCOLATE SYRUP, WHOLE MILK ADDED	75
11513550	CHOCOLATE SYRUP, RED FAT MILK ADDED	75
11513600	CHOCOLATE SYRUP, LOWFAT MILK ADDED	75
11513700	CHOCOLATE SYRUP, SKIM MILK ADDED	75
11514100	COCOA, SUGAR, & DRY MILK MIXTURE, WATER ADDED	75
11514300	COCOA W/ NF DRY MILK, LO CAL SWEETENER, WATER ADDED	75
11518050	MILK BEV W/NF DRY MILK, LO CAL SWEET,WATER,NOT CHOC	75
11518100	MILK BEV W/NFD MILK, LO CAL SWEET, HI CALCIUM, CHOC	75
11519000	MILK BEVERAGE, NOT CHOCOLATE, W/ WHOLE MILK	75
11519050	MILK, NOT CHOCOLATE, WHOLE MILK BASED	75
11520000	MILK, MALTED, UNFORTIFIED, FLAVOR NS	75
11525000	MILK,MALTED,FORTIFIED,NATURAL FLAVOR (INCL	75
11526000	MILK, MALTED, FORTIFIED, CHOCOLATE (INCL OVALTINE)	75
11527000	MILK, MALTED, FORTIFIED, (INCL OVALTINE)	75
11531000	EGGNOG, MADE W/ WHOLE MILK (INCLUDE EGG NOG, NFS)	75
11541000	MILK SHAKE, NS AS TO FLAVOR OR TYPE	75
11541110	MILK SHAKE, HOMEMADE OR FOUNTAIN-TYPE, CHOCOLATE	75
11541120	MILK SHAKE, HOMEMADE/FOUNTAIN-TYPE, NOT CHOCOLATE	75
11541400	MILK SHAKE WITH MALT (INCL MALTED MILK W/ICE CREAM)	75
11541500	MILK SHAKE, MADE W/ SKIM MILK, CHOCOLATE	75
11541510	MILK SHAKE,MADE W/ SKIM MILK, NOT CHOCOLATE	75
11542100	CARRY-OUT MILK SHAKE, CHOCOLATE	75
11542200	CARRY-OUT MILK SHAKE, NOT CHOCOLATE	75
11551050	MILK FRUIT DRINK (INCL LICUADO)	75
11552200	MILK-BASED FRUIT DRINK (INCL ORANGE JULIUS)	75
11553000	FRUIT SMOOTHIE DRINK, W/ FRUIT AND DAIRY PRODUCTS	75
11561010	CAFE CON LECHE PREPARED W/ SUGAR	75
11611000	INSTANT BREAKFAST, FLUID, CANNED	75
11612000	INSTANT BREAKFAST, POWDER, MILK ADDED	75
11613000	INSTANT BFAST,PWDR,SWT W/ LO CAL SWT, MILK ADDED	75
11810000	MILK, DRY, NOT RECONSTITUTED, NS AS TO FAT	100
11811000	MILK, DRY, WHOLE, NOT RECONSTITUTED	100
11812000	MILK, DRY, LOWFAT, NOT RECONSTITUTED	100
11813000	MILK, DRY, NONFAT, NOT RECONSTITUTED	100
11830100	COCOA W/DRY MILK & SUGAR, DRY MIX, NOT RECONST	75
11830110	COCOA POWDER W/ NFD MILK, LOW CAL SWEETENER, DRY	75
11830160	COCOA-FLAVORED BEVERAGE POWDER W/ SUGAR, DRY MIX	75
11830180	COCOA-FLAV BEV MIX, LOW CALORIE, DRY, NOT RECONST	75
11830200	MILK, MALTED, DRY, UNFORTIFD, NOT RECONST, NOT CHOC	75
11830210	MILK, MALTED, DRY, FORTIFD, NOT RECONST, NOT CHOC	75
11830250	MILK, MALTED, DRY, UNFORTIFIED, NOT RECONST, CHOC	75
11830260	MILK, MALTED, DRY, FORTIFIED, NOT RECONST, CHOC	75
11830400	MILK BEV POWDER, DRY, NOT RECONST, NOT CHOC	75
11830800	INSTANT BREAKFAST POWDER, NOT RECONSTITUTED	75
11830810	INSTANT BFAST,PWDR,SWT W/ LO CAL SWT,NOT RECONSTUT	75
12100100	CREAM, FLUID, NS AS TO LIGHT, HEAVY OR HALF & HALF	100

12110100	CREAM, LIGHT, FLUID (INCL COFFEE CRM, TABLE CREAM)	100
12110300	CREAM, LIGHT, WHIPPED, UNSWEETENED	100
12120100	CREAM, HALF & HALF	100
12130100	CREAM, HEAVY, FLUID	100
12140000	CREAM, HEAVY, WHIPPED, SWEETENED	100
12140100	CREAM, WHIPPED, PRESSURIZED CONTAINER	100
12310100	SOUR CREAM (INCL W/ CHIVES)	100
12310200	SOUR CREAM, HALF & HALF	100
12310300	SOUR CREAM, REDUCED FAT	75
12310350	SOUR CREAM, LIGHT	75
12310370	SOUR CREAM, FAT FREE	75
12320200	SOUR CREAM, FILLED, SOUR DRESSING, NONBUTTERFAT	75
12350000	DIP, SOUR CREAM BASE (INCLUDE BUTTERMILK-TYPE DIP)	75
12350020	DIP, SOUR CREAM BASE, REDUCED CALORIE	75
12350100	SPINACH DIP, SOUR CREAM BASE	75
13110000	ICE CREAM, NFS	75
13110100	ICE CREAM, REGULAR, NOT CHOCOLATE	75
13110110	ICE CREAM, REGULAR, CHOCOLATE	75
13110120	ICE CREAM, RICH, FLAVORS OTHER THAN CHOCOLATE	75
13110130	ICE CREAM, RICH, CHOCOLATE	75
13110140	ICE CREAM, RICH, NS AS TO FLAVOR	75
13110200	ICE CREAM, SOFT SERVE, NOT CHOCOLATE	75
13110210	ICE CREAM, SOFT SERVE, CHOCOLATE	75
13110220	ICE CREAM, SOFT SERVE, NS AS TO FLAVOR	75
13110400	MILK DESSERT, FRZN, FLAVORS NOT CHOC (NO BUTTERFAT)	75
13110450	MILK DESSERT, FROZEN, CHOCOLATE (NO BUTTERFAT)	75
13120050	ICE CREAM BAR OR STICK, NOT CHOC- OR CAKE-COVERED	75
13120100	ICE CREAM BAR/STICK, CHOCOLATE COVERED	75
13120110	ICE CREAM BAR, CHOCOLATE/CARAMEL COVERED, W/ NUTS	75
13120120	ICE CREAM BAR,RICH CHOC ICE CREAM,THICK CHOC COVER	75
13120121	ICE CREAM BAR,RICH ICE CREAM,THICK CHOC COVER	75
13120130	ICE CREAM BAR/STICK,RICH ICE CREAM,CHOC COVER,W/NUT	75
13120140	ICE CREAM BAR/STICK, CHOC ICE CREAM, CHOC COVER	75
13120300	ICE CREAM BAR, CAKE-COVERED	75
13120400	ICE CREAM BAR/STICK W/ FRUIT	75
13120500	ICE CREAM SANDWICH	75
13120550	ICE CREAM COOKIE SANDWICH (INCLUDE CHIPWICH)	75
13120700	ICE CREAM CONE, W/ NUTS, NOT CHOCOLATE	75
13120710	ICE CREAM CONE, CHOC-COVERED, W/ NUTS, NOT CHOC	75
13120720	ICE CREAM CONE, CHOC-COVERED OR DIPPED, NOT CHOC	75
13120730	ICE CREAM CONE, NO TOPPING, NOT CHOCOLATE	75
13120740	ICE CREAM CONE, NO TOPPING, NS AS TO FLAVOR	75
13120750	ICE CREAM CONE, W/NUTS, CHOCOLATE ICE CREAM	75
13120770	ICE CREAM CONE, NO TOPPING, CHOCOLATE ICE CREAM	75
13120790	ICE CREAM SUNDAE CONE (INCL DRUMSTICK, ALL FLAVORS)	75
13120800	ICE CREAM SODA, NOT CHOCOLATE	75
13120810	ICE CREAM SODA, CHOCOLATE	75

13121100	ICE CREAM SUNDAE, FRUIT TOPPING, W/ WHIPPED CREAM	75
13121200	ICE CREAM SUNDAE, PREPACKAGED, NOT CHOCOLATE	75
13121300	ICE CREAM SUNDAE,CHOCOLATE TOPPING,W/ WHIPPED	75
13121500	ICE CREAM SUNDAE, FUDGE TOPPING, W/ CAKE	75
13122100	ICE CREAM PIE, NO CRUST	75
13122500	ICE CREAM PIE,COOKIE CRUST,FUDGE TOPPING,WHIP CREAM	75
13124100	SORBET & ICE CREAM	75
13125100	ICE CREAM W/ SHERBET	75
13126000	ICE CREAM, FRIED	75
13130100	LIGHT ICE CREAM,NFS (FORMERLY ICE MILK)	75
13130300	LIGHT ICE CREAM,NOT CHOCOLATE (FORMERLY ICE MILK)	75
13130310	LIGHT ICE CREAM,CHOCOLATE (FORMERLY ICE MILK)	75
13130350	LIGHT ICE CREAM,PREMIUM, NOT CHOC (FORMERLY ICE	75
13130360	LIGHT ICE CREAM,PREMIUM, CHOCOLATE (FORMERLY ICE	75
13130590	LIGHT ICE CREAM,SOFT SERVE, NS FLAVOR (FORMERLY ICE	75
13130600	LIGHT ICE CREAM,SOFT SERVE, NOT CHOC (FORMERLY ICE M	75
13130610	LIGHT ICE CREAM,SOFT SERVE CHOC (TASTEE FRZ, DAIRY Q	75
13130620	LIGHT ICE CREAM,SOFT SERVE CONE,NOT CHOC (DAIRY QUEE	75
13130630	LIGHT ICE CREAM,SOFT SERVE CONE, CHOC (FORMERLY ICE	75
13130640	LIGHT ICE CREAM,SOFT SERVE CONE, NS FLAV(FORMERLY IC	75
13135000	LIGHT ICE CREAM,SANDWICH (DAIRY QUEEN) (FORMERLY	75
13140100	LIGHT ICE CREAM,BAR/STICK, CHOC-COATED (FORMERLY ICE	75
13140110	LIGHT ICE CREAM,BAR, CHOC COVERED,W/NUTS (FORMERLY	75
13140550	LIGHT ICE CREAM,CONE, CHOCOLATE (FORMERLY ICE MILK)	75
13140600	LIGHT ICE CREAM,SUNDAE,SOFT SERVE,CHOC/FUDGE TOP (IC	75
13140630	LIGHT ICE CREAM,SUNDAE,SOFT SERVE,FRUIT TOPPING (ICE	75
13140660	LIGHT ICE CREAM,SUNDAE,CHOC / FUDGE TOP (W/O WHIP CR	75
13140670	LIGHT ICE CREAM,SUNDAE,FRUIT TOP (W/O WHIP CREAM)(IC	75
13140680	LIGHT ICE CREAM,SUNDAE,NO FRUIT/CHOC TOP (W/O WHIP C	75
13140700	LIGHT ICE CREAM,CREAMSICLE OR DREAMSICLE (FORMERLY	75
13140900	LIGHT ICE CREAM,FUDGESICLE (FORMERLY ICE MILK)	75
13141100	LIGHT ICE CREAM,W/ SHERBET OR ICE CREAM (FORMERLY IC	75
13142000	MILK DESSERT BAR/STICK, FROZEN, W/ COCONUT	75
13150000	SHERBET, ALL FLAVORS	75
13160100	MILK DESSERT,FZN,LOWFAT,W/LOW CAL SWEET,NOT CHOC	75
13160150	MILK DESSERT, FROZ, NONFAT, W/ LOW CAL SWEETENER	75
13160160	MILK DESSERT, FROZ, NONFAT, W/ LOW CAL SWEETENER, NO	75
13160200	MILK DESSERT,FROZEN,LOWFAT,NOT CHOCOLATE	75
13160210	MILK DESSERT, FROZEN, LOWFAT, CHOCOLATE	75
13160400	MILK DESSERT, FROZ, MILK-FAT FREE, NOT CHOC	75
13160410	MILK DESSERT, FROZ, MILK-FAT FREE, CHOC	75
13160600	MILK DESSERT, FROZ, W/ LOW CAL SWEETENER, NOT CHOC	75
13160650	MILK DESSERT, FROZ, W/ LOW CAL SWEETENER, CHOCOLATE	75
13161000	MILK DESSERT BAR, FROZEN, MADE FROM LOWFAT MILK	75
13161600	MILK DES BAR, FROZEN, LOFAT MILK&LO CAL SWEETENER	75
13161630	LIGHT ICE CREAM,BAR/STICK, W/ LOW-CAL SWEETENER, CHO	75
13200110	PUDDING, NFS	75

13210220	PUDDING, CHOCOLATE, RTE, NS FROM DRY MIX OR CANNED	75
13210250	PUDDING, CHOC, RTE, LO CAL, W/ ART SWTNER, NS DRY/CA	75
13210280	PUDDING, NOT CHOC, RTE, NS FROM DRY MIX OR CANNED	75
13210290	PUDDING, NOT CHOC, RTE, LO CAL, W/ ART SWTNER	75
13210410	PUDDING, RICE	75
13210450	PUDDING, RICE FLOUR, W/ NUTS (INDIAN DESSERT)	75
13210500	PUDDING, TAPIOCA,MADE FROM HOME RECIPE, MADE W/	75
13210520	PUDDING, TAPIOCA,MADE FROM DRY MIX,MADE W/ MILK	75
13210530	PUDDING, TAPIOCA,CHOCOLATE,MADE W/ MILK	75
13210610	PUDDING, COCONUT	75
13210710	PUDDING, INDIAN (MILK, MOLASSES, CORNMEAL-BASED)	75
13210750	PUDDING, PUMPKIN	75
13220110	PUDDING,NOT CHOCOLATE,PREPARED FROM DRY MIX,MILK	75
13220120	PUDDING,CHOCOLATE,PREPARED FROM DRY MIX,MILK	75
13220210	PUDDING,NOT CHOC,FROM DRY,LOW CAL,ARTIFICIAL	75
13220220	PUDDING,CHOC,FROM DRY,LOW CAL,ARTIFICIAL	75
13220230	PUDDING, CAN, CHOC, REDUCED FAT (INCL JELL-O LIGHT)	75
13220235	PUDDING, CAN, CHOC, FAT FREE	75
13220240	PUDDING, CANNED, NOT CHOC, REDUCED FAT	75
13220245	PUDDING, CANNED, NOT CHOC, FAT FREE	75
13230110	PUDDING, CANNED, NOT CHOCOLATE	75
13230120	PUDDING,CANNED,LOW CAL,W/ARTIFICIAL SWTNR,NOT	75
13230130	PUDDING, CANNED, CHOCOLATE	75
13230140	PUDDING, CANNED, LO CAL, W/ ART SWTNER, CHOC	75
13230200	PUDDING, CANNED, CHOC & NON-CHOC FLAVORS COMBINED	75
13230500	PUDDING, CANNED, TAPIOCA	75
13230510	PUDDING, CANNED, TAPIOCA, FAT FREE	75
13235000	PUDDING, POPS, CHOCOLATE	75
13241000	PUDDING, W/ FRUIT & VANILLA WAFERS	75
13250000	MOUSSE, CHOCOLATE	75
13250100	MOUSSE, NOT CHOCOLATE	75
13252100	COCONUT CUSTARD, P.R. (FLAN DE COCO)	75
13252200	MILK DESSERT OR MILK CANDY, P.R. (DULCE DE LECHE)	75
13252500	BARFI/BURFI,INDIAN DESSERT,FROM MILK/CREAM/RICOTTA	75
13310000	CUSTARD PUDDING, NOT CHOC, BABY, NS AS TO STR OR JR	75
13311000	CUSTARD PUDDING, BABY, NOT CHOCOLATE, STRAINED	75
13312000	CUSTARD PUDDING, BABY, NOT CHOCOLATE, JUNIOR	75
13411000	WHITE SAUCE, MILK SAUCE	75
13412000	MILK GRAVY, QUICK GRAVY	75
92101900	COFFEE, LATTE	100
92161000	CAPPUCCINO	100
92162000	CAPPUCCINO, DECAFFEINATED	100
93301180	MIXED DRINKS (FOR RECIPE MODIFICATIONS)	100
93301250	WHITE RUSSIAN	100
93301400	IRISH COFFEE (INCL COFFEE ROYALE)	100
93301450	LIQUEUR W/ CREAM	100

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Appendix B: Food codes in the 1994-96, 1998 Continuing Survey of Food Intakes by Individuals that are representative of the proposed uses of bovine lactoferrin in functional foods

Use Category [a]	Food Code	Food Name	Serving Size (g) [b]
RTD Bev	11611000	INSTANT BREAKFAST, FLUID, CANNED	313
RTD Bev	11621000	DIET BEVERAGE, LIQUID, CANNED	313
RTD Bev	11623000	MEAL SUPPLEMENT / REPLACEMENT,PREPARED,RTD	341
RTD Bev	11631000	HIGH CALORIE BEV, CANNED OR POWDERED, RECONSTITUTED	399
RTD Bev	11641000	MEAL REPLACEMENT, MILK BASED, HIGH PROTEIN, LIQUID	256
RTD Bev	41440010	MEAL REPLACEMENT/SUPPLEMENT, LIQUID, HI PROTEIN	252
RTD Bev	41440020	ENSURE W/ FIBER, LIQUID	248
RTD Bev	41440050	ENSURE PLUS LIQUID NUTRITION	252
RTD Bev	41440100	MEAL REPLACEMENT,LIQUID,SOY-BASE (ISOCAL, OSMOLITE)	370
Bev mix	11612000	INSTANT BREAKFAST, POWDER, MILK ADDED	279
Bev mix	11613000	INSTANT BFAST,PWDR,SWT W/ LO CAL SWT, MILK ADDED	247
Bev mix	11830800	INSTANT BREAKFAST POWDER, NOT RECONSTITUTED	37
Bev mix	11830810	INSTANT BFAST,PWDR,SWT W/ LO CAL SWT,NOT RECONSTUT	20
Bev mix	11830850	HIGH CALORIE MILK BEVERAGE, POWDER, NOT RECONST	30
Bev mix	11830900	PROTEIN SUPPLEMENT, MILK BASED, DRY POWDER	43
Bev mix	11830940	MEAL REPLACEMENT,PROTEIN,MILK BASED,FRUIT JUICE MIX	31
Bev mix	11830970	MEAL REPLACEMENT, PROTEIN TYPE, MILK-BASE, POWDER	33
Bev mix	11830990	NUTRIENT SUPP, MILK-BASE, POWDER (INCL SUSTAGEN)	31
Bev mix	11831500	NUTRIENT SUPPLEMENT,MILK-BASE,HIGH PROT,NOT RECONST	27
Bev mix	11832000	MEAL REPLACEMENT,MILK-&SOY-BASE,POWDER,NOT RECONST	44
Bev mix	11835100	MEAL REPLACEMENT, POSITRIM DRINK MIX, DRY POWDER	43
Bev mix	11835200	LOSE-IT (NANCI), MEAL REPLACEMENT, POWDER	21
Bev mix	41430000	PROTEIN POWDER, NFS	28
Bev mix	41430010	PROTEIN SUPPLEMENT, POWDERED	28
Bev mix	41430100	FORMULATED DIET MEAL, POWDER, SOY PROTEIN ISOLATE	28.35
Bev mix	41430200	MEAL REPLACE / SUPP, SOY-MILK-BASE,POWD,WATER ADDED	218
Bev mix	41430310	PROTEIN DIET POWDER W/ SOY & CASEIN	20
Bar	41435110	HIGH PROTEIN BAR, CANDY-LIKE, SOY & MILK BASE	50
Bar	53541100	BREAKFAST BAR, DIET MEAL TYPE	25
Bar	53541200	MEAL REPLACEMENT BAR (INCL SLIM FAST BAR)	34
Bar	53544450	POWERBAR (FORTIFIED HIGH ENERGY BAR)	65

[a] Bev mix = Beverage mixes; RTD Bev = Ready-To-Drink Beverage
[b] Default serving sizes (in grams) for each of the food codes were identified using the USDA Survey Food Coding Database (USDA, 2000).

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January 15, 2001

Dr. Paulette Gaynor
Office of Premarket Approval, HFS-200
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, SW
Washington, DC 20204

Dear Dr. Gaynor:

We wish to notify you of a typographical error in the January 12, 2001 addendum to the GRAS Notification for bovine lactoferrin for use as an ingredient in sports and functional foods, as described in GRN No. 000042.

We have enclosed copies of the two pages (pages 3 and 5) on which the error occurs and is highlighted, as well as corrected copies of these two pages. We ask that you replace pages 3 and 5 of your document with the corrected pages. None of the calculations presented in the document are affected by this change.

Sincerely,

Claire L. Kruger, Ph.D., D.A.B.T.
Senior Science Manager

cc: Bill Frankos
Ram Nimmagudda

Attachments

000465

4350 North Fairfax Drive • Suite 300 • Arlington, Virginia 22203 • USA • Tel: (703) 516-2300 • Fax: (703) 516-2345
www.environcorp.com

Background Exposure to Bovine Lactoferrin from Dairy Products and Whey Products Added to Food

Bovine lactoferrin has been consumed by humans for thousands of years as a naturally occurring protein found in milk. Current sources of lactoferrin in the American diet include milk proteins naturally occurring in milk and milk products, and whey proteins added to foods throughout the food supply in the form of whey products. Milk proteins and whey proteins contain approximately 0.03 g and 1.4 g lactoferrin per 100 g, respectively (Barth and Behnke 1997).

Using food intake data reported in the United States Department of Agriculture's (USDA) 1994-96 Continuing Survey of Food Intakes by Individuals (CSFII) and its 1998 Supplemental Children's Survey (USDA 2000), ENVIRON determined the average per capita intake of milk proteins from milk and milk products (Table 1).

The CSFII 1994-96 was conducted between January 1994 and January 1997 with non-institutionalized individuals in the United States. In each of the three survey years, data were collected from a nationally representative sample of individuals of all ages. The CSFII 1998 was a survey of children ages 0 through 9 which was supplemental to the CSFII 1994-96. It used the same sample design as the CSFII 1994-96 and was intended to be merged with CSFII 1994-96 to increase the sample size for children. The merged surveys are designated as CSFII 1994-96, 1998. In the CSFII 1994-96, 1998, dietary intakes were collected through in-person interviews using 24-hour recalls on two nonconsecutive days approximately one week apart. A total of 21,662 individuals provided data for the first day; of those individuals, 20,607 provided data for a second day. The survey database includes data on 52 dietary components, including protein, for each food reported.

The estimates of milk protein intake were based on reported consumption of all milk and milk products including fluid and flavored milk, dry and reconstituted milk, milk-based beverage mixes, milk and milk products in beverage mixtures, yogurt, ice cream, cream, sour cream, and puddings. The analyses did not include cheese, infant formulas, meal replacements, whey-based beverages and beverage mixes, or butter. Additionally, the milk and milk products that were ingredients in food mixtures coded as a single item (i.e., milk used in the preparation of canned soup) were not included in the estimates. Given that whey proteins may be added to some milk products such as yogurt, ice cream, and beverage mixes, we assumed only 75 percent of the protein content of milk products to be milk protein; this allows for the addition of approximately one gram of whey protein per 100 grams of each milk product. The food codes included in the estimates of milk protein intake are listed in Appendix A.

The mean intake of milk proteins by individuals ages 2 years and older in the U.S. is approximately 7.7 g per day. This is equivalent to approximately 23 mg lactoferrin per day. The 90th percentile of milk protein intake in the U.S. is approximately 18.6 g per day, or 56 mg lactoferrin per day. The 95th percentile of milk protein intake is 23.5 g per

TABLE 1
Estimate of Per Capita Daily
Intake of Bovine Lactoferrin from Milk Proteins and Whey Proteins

	Milk Proteins		Whey Proteins		Milk & Whey Proteins
	Milk Proteins in Dairy Products [a] (g)	Lactoferrin from Milk Proteins in Dairy Products [a, b] (mg)	Whey Proteins in the Food Supply [c] (g)	Lactoferrin from Whey Proteins in the Food Supply [c, d] (mg)	Lactoferrin from Milk and Whey Proteins [e] (mg)
Mean Intake	7.7	23	1.0	14	37
90th Percentile of Intake	18.6	56	2.0	28	84
95th Percentile of Intake	23.5	70	2.0 [f]	28 [f]	98

[a] Data source: USDA 1994-96, 1998 Continuing Survey of Food Intakes by Individuals; estimates reflect intake of protein from milk and milk products including fluid and flavored milk, dry and reconstituted milk, milk-based beverage mixes, yogurt, ice cream, cream, sour cream, and puddings. Estimates include all individuals 2 years and older who provided two 24-hour diet recalls. Estimates generated with USDA sampling weights and WesVar Complex Samples Version 3.0.
[b] Calculation of lactoferrin based on 0.03% lactoferrin in milk protein (Barth and Behnke 1997).
[c] Data source: FDA estimate of per capita whey products consumption (Rulis 2000; Agency Response Letter to GRAS Notice No. GRN 000037); assumed 25% whey protein in whey products; assumed 90th percentile of intake is twice the mean intake.
[d] Calculation of lactoferrin based on 1.4% lactoferrin in whey protein (Barth and Behnke 1997).
[e] Represents sum of estimates of lactoferrin from milk proteins in dairy products and lactoferrin from whey proteins in the food supply.
[f] Unable to estimate 95th percentile; values represent estimate of 90th percentile.

BLANK PAGE INSERTED FOR CORRECT PAGINATION

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April 6, 2001

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OFFICE OF
PREMARKET APPROVAL

Dr. Paulette Gaynor
Office of Premarket Approval, HFS-200
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, SW
Washington D.C. 20204

Dear Dr.Gaynor:

We received your letter of March 26 in response to GRAS Notice No. GRN 000042. The subject of this GRAS Notice is bovine milk-derived lactoferrin. Scientific procedures were used to evaluate the safety and GRAS status of milk-derived lactoferrin for use a nutrient supplement in sports and functional foods.

An addendum to GRN 000042 was submitted on January 12, 2001. This addendum provided a revised calculation of the background exposure to milk-derived lactoferrin through dairy products and whey products added to food. This calculation indicates that background exposure can be as high as 100 mg lactoferrin per person per day. This background exposure is consistent with intake by individuals who consume dairy products at the level recommended by the United States Department of Agriculture (USDA) Food Guide Pyramid. A revised calculation of the estimated daily intake (EDI) of milk-derived lactoferrin from its proposed uses was also included in the submitted addendum. This revised EDI indicates that mean consumption of milk-derived lactoferrin is 105 mg/person/day. The proposed EDI will result in no more than a doubling of potential background exposure. In addition, products containing the DMV milk-based lactoferrin would be labeled as containing a milk-derived product and would not be expected to be consumed by those individuals with a milk allergy.

The FDA letter of March 26 has identified two remaining issues that need to be addressed to support the GRAS determination of milk-based lactoferrin at the level of exposure indicated above. These issues are (1) the potential that the increased level and overall exposure to milk-derived lactoferrin would sensitize individuals who are not already allergic (to milk) and (2) the potential that this immunologically active food component is correlated with autoimmune disorders. FDA has indicated that DMV International must provide the basis to conclude that experts have considered these issues and agree that the available data and information demonstrate that the potential for allergenicity or autoimmune problems does not raise a safety concern for milk-based lactoferrin at the levels and uses proposed by DMV International.

It is our understanding that the two issues described above are the only remaining issues that need to be addressed to complete the GRAS determination of milk-based lactoferrin. ENVIRON is convening a panel of experts to evaluate the safety of milk-derived

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www.environcorp.com

lactoferrin with respect to potential for allergenicity and autoimmunity. Please find attached a summary letter from Dr. Steven Taylor summarizing his initial review of the issues. The Expert Panel will meet as quickly as possible. After the meeting, a summary of the conclusions reached by that Panel will be sent to FDA. The Panel conclusions will supplement information already provided to FDA that provide the basis for the conclusion reached by DMV International that milk-based lactoferrin is GRAS for the intended uses set forth in GRN000042 and the addendum of January 12, 2001.

Sincerely,

Claire L. Kruger, Ph.D., D.A.B.T.
Senior Science Manager

Enclosure

cc: Dr. Alan Rulis

INITIAL EXPERT OPINION ON LACTOFERRIN

April 6, 2001

Steve L. Taylor, Ph.D.
Lincoln, NE 68510

The Food & Drug Administration has objected to the designation of bovine lactoferrin as GRAS on the basis of concerns about the possibility that lactoferrin might be or become an allergen and the possibility that ingestion of lactoferrin might induce autoimmune responses. These concerns were first raised by FDA officials in a meeting with DMV Nutritionals in October 2000. I had advised DMV Nutritionals at that time, that, in my expert opinion, these concerns were not significant especially given the comparative exposure estimates for the new uses of lactoferrin versus existing exposure through ingestion of milk and other milk-derived products. However, recent correspondence from FDA indicates that the comparative exposure information alone is not sufficiently persuasive and that FDA wishes to obtain more information on two concerns.

The standard for a GRAS determination is reasonable certainty of no harm. This standard should apply to concerns about abnormal immunological responses just as it does to other toxicological endpoints. Furthermore, the situation with lactoferrin must be placed in context with previous decisions that have been made regarding protein-based ingredients because FDA will likely be faced with other protein-based ingredients in the future. In this context, casein and whey are considered GRAS food ingredients even though casein, β-lactoglobulin, and α-lactalbumin are the major allergens in cows' milk. The widespread use of casein and whey as food ingredients has certainly increased consumer exposure to these allergens in a rather significant way over the years.

Due to FDA's remaining questions regarding the safety of lactoferrin, I have now recommended to DMV Nutritionals that they convene an Expert Panel of medical and scientific experts in allergy and immunology to review the pertinent scientific literature on lactoferrin and to provide their collective expert opinion on the safety of lactoferrin as a food ingredient. Obviously, some time will be required for DMV to act upon this recommendation. In the meantime, allow me to present my current expert opinion on these two scientific issues while urging FDA to keep the existing DMV file open while awaiting this additional expert input.

Lactoferrin as a Food Allergen

Current clinical information is insufficient, in my opinion, to conclude that lactoferrin is a milk allergen. Certainly, there is no argument that the major cows' milk allergens in IgE-mediated cows' milk allergy are casein, β-lactoglobulin, and α-lactalbumin (Hefle et al., 1996; Wal, 1998). Several clinical investigators have demonstrated that some cows'

milk-allergic individuals have IgE antibodies directed against lactoferrin (Baldo, 1984; Host et al., 1992; Wal et al., 1995; Wal, 1998). However, the vast majority of these milk-allergic infants displayed IgE antibodies to one or more of the major cows' milk allergens in addition to lactoferrin. Wal (1998) appears to have identified one milk-allergic infant out of 92 studied who was mono-sensitized to lactoferrin only. However, the clinical significance of this single observation is unclear because the study does not indicate if this or other infants in the study had received blinded oral challenges to document their milk allergies. Without confirmation by oral challenge, the evaluation of specific IgE antibodies in patient serum can be clinically meaningless. Furthermore, in studies where quantitative estimates were obtained of the levels of specific IgE antibodies to lactoferrin (Wal et al., 1995; Wal, 1998), the levels of anti-lactoferrin IgE antibodies were far lower than the levels of specific IgE antibodies to the major milk allergens in the vast majority of the patients. Milk-allergic patients seem to occasionally have IgE antibodies directed at other minor milk proteins (Baldo, 1984); the clinical significance of these antibodies is unproven. To demonstrate that lactoferrin is a clinically significant milk allergen, patients who were challenge-positive to milk ingestion would have to demonstrate specific IgE antibodies to lactoferrin (and possibly other milk proteins), positive skin prick tests to lactoferrin, and positive oral challenges to lactoferrin. Such information does not exist to demonstrate that lactoferrin is an allergen.

Would lactoferrin become an allergen if increased levels of lactoferrin exposure occurred in the diet? First of all, the current DMV submission clearly indicates that their new uses for lactoferrin would not significantly increase consumer exposure to lactoferrin. But, studies in Brown Norway rats conducted by the BIBRA group in the U.K. indicate that lactoferrin can induce IgE responses in these rats when injected intraperitoneally (Atkinson and Miller, 1994; Miller et al., 1998). However, this particular Brown Norway rat model is not particularly appropriate for the prediction of human responses to ingestion of lactoferrin or other proteins. First, this model has not been validated. It does not discriminate between allergenic and non-allergenic proteins. No protein ever tested in this model with I.P. injection has ever been negative. In my opinion, the predictive value of this study is essentially zero. In further studies with the Brown Norway rat using oral exposures, lactoferrin did not induce an IgE response (Meredith and Atkinson, 2000; Meredith and Atkinson, 2001), a result that raises further questions regarding the initial observations.

Therefore, my current opinion is that lactoferrin is not a documented cows' milk allergen and there is no proof to indicate that it will become an allergen should exposure increase through its use as a food ingredient.

Lactoferrin and Autoimmune Disease

The hypothesis has been proposed that exposure to bovine lactoferrin in the diet might promote a subsequent autoimmune response to human lactoferrin (Brock et al., 1998). The evidence supporting this hypothesis is very meager in my opinion. In autoimmune disease, human lactoferrin generated from neutrophils definitely acts as one of the autoantigen. In the Brock et al. study, IgG antibodies to human lactoferrin were elevated

in most ANCA+ (autoimmune disease marker) patients. The sera of these same patients had elevated IgG antibodies to bovine lactoferrin. However, since human and bovine lactoferrin share about 70% homology, this finding could represent simply cross-reactivity of antigen-antibody binding that has no clinical significance. So, this finding may simply corroborate the known increase in IgG antibodies to human lactoferrin that occurs in autoimmune disease. Furthermore, the demonstration that ingestion of bovine lactoferrin resulted in increased IgG antibodies to both bovine and human lactoferrin in some infants is not unexpected because such IgG responses are commonly encountered to dietary proteins especially in infants with compromised gastrointestinal barriers. Such IgG increases are often considered a form of protection against the entry of dietary antigens. But, the presence of these IgG antibodies does not indicate that autoimmune disease will result. Certainly, this study does not prove that ingestion of bovine lactoferrin will lead to an increase in autoimmune disease. At best, it is a hypothesis-generating study that likely applies only to infants in any case.

The digestive fate of ingested lactoferrin seems to be a crucial factor in respect to any potential concerns about a possible effect on autoimmune disease. Bovine and human lactoferrin are only slowly and partially degraded in the intestinal tract in young infants (Davidson and Lonnerdal, 1985; Spik et al., 1982); bovine lactoferrin appears to be somewhat more resistant to proteolysis than human lactoferrin (Spik et al., 1982). However, digestion of lactoferrin becomes more efficient as the infant ages. By 15 weeks of age, there are no differences in plasma lactoferrin between adults and infants (Scott, 1989). The plasma lactoferrin in adults is human lactoferrin derived from neutrophils because ingested lactoferrin is rapidly digested and/or sequestered in the liver and spleen and catabolized there (Bennett and Kokocinski, 1979). Therefore, the addition of bovine lactoferrin to the diets of adults would be predicted to have little effect on plasma lactoferrin levels which would seem to eliminate concerns about its role in autoimmune disease.

Thus far, the findings are not convincing that ingestion of bovine lactoferrin play a role in the onset of autoimmune disease especially in adults.

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NA- Not applicable