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FOOD ADDITIVE SAFETY

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July 15, 2016

VIA FEDERAL EXPRESS

Dr. Antonia Mattia
Director
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: GRAS Notification for the Lactoperoxidase System

Dear Dr. Mattia:

On behalf of Taradon Laboratory ("Taradon"), we are submitting under cover of this letter three paper copies and one eCopy of DSM's generally recognized as safe ("GRAS") notification for its lactoperoxidase system ("LPS"). The electronic copy is provided on a virus-free CD, and is an exact copy of the paper submission. Taradon has determined through scientific procedures that its lactoperoxidase system preparation is GRAS for use as a microbial control adjunct to standard dairy processing procedures such as maintaining appropriate temperatures, pasteurization, or other antimicrobial treatments to extend the shelf life of the products.

In many parts of the world, the LPS has been used to protect dairy products, particularly in remote areas where farmers are not in close proximity to the market. In the US, the LPS is intended to be used as a processing aid to extend the shelf life of a variety of dairy products, specifically fresh cheese including mozzarella and cottage cheeses, frozen dairy desserts, fermented milk, flavored milk drinks, and yogurt. The Lactoperoxidase system is a natural defense system against microbial contamination. The LPS has been reviewed by a number of international organizations, including WHO, because of its use in remote areas for the treatment of milk products. All of the components of the LPS system occur naturally in human and animal liquid secretions, and therefore presents no new exposures to the human body. The system provides antimicrobial activity against a wide spectrum of spoilage and pathogenic microorganisms.

Pursuant to the regulatory and scientific procedures established by proposed regulation 21 C.F.R. § 170.36, this use of the lactoperoxidase is exempt from premarket approval requirements of the Federal Food, Drug and Cosmetic Act, because the notifier has determined that such use is GRAS.

If you have any questions regarding this notification, or require any additional information to aid in the review of Taradon's conclusion, please do not hesitate to contact me via email at gary.yingling@morganlewis.com or by telephone, (202)739-5610.

Sincerely,

(b) (6)

Gary L. Yingling

cc: Taradon Laboratory

GRAS NOTIFICATION FOR THE LACTOPEROXIDASE SYSTEM

Submitted by:
Taradon Laboratory
Avenue Leon Champagne, 2
B-1480 Tubize
Belgium

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1. GENERAL INTRODUCTION AND CLAIM OF EXEMPTION FROM PREMARKET APPROVAL REQUIREMENTS

1.1. Name and Address of Notifier

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1.2. Common or Usual Name of Substance

Lactoperoxidase System (LPS) is the proposed common or usual name of the mixture. The proposed trade name will be Taradon LPO System

1.3. Applicable Conditions of Use

The LPS is intended to be used as a microbial control adjunct to standard dairy processing procedures such as maintaining appropriate temperatures, pasteurization, or other antimicrobial treatments to extend the shelf life of the products. The use of the LPS in dairy products reduces microbial activity, and thus reduces the incidence of food-borne illness.

1.3.1. Substances Used In

The LPS is intended for use in fresh cheese including mozzarella and cottage cheeses, frozen dairy desserts, fermented milk, flavored milk drinks, and yogurt.

1.3.2. Levels of Use

The LPS is intended for use at a level of 300 mg/L milk used to produce the substances listed above in 1.3.1. The formulation for LPS is as follows:

Lactoperoxidase: 1.25%
Glucose oxidase: 0.75%
Glucose: 30%
Sodium Thiocyanate: 5%
Sucrose: 63%

1.3.3. Purposes

In many parts of the world, the LPS has been used to protect dairy products, particularly in remote areas where farmers are not in close proximity to the market. In the US, the LPS is intended to be used as a processing aid to extend the shelf life of a variety of dairy products, specifically fresh cheese including mozzarella and cottage cheeses, frozen dairy desserts, fermented milk, flavored milk drinks, and yogurt. The Lactoperoxidase system is a natural defense system against microbial contamination. The LPS has been reviewed by a number of international organizations, including WHO, because of its use in remote areas for the treatment of milk products. As will be explained, all of the components of the LPS system occur naturally in human and animal liquid secretions, and therefore presents no new exposures to the human body. The system provides antimicrobial activity against a wide spectrum of spoilage and pathogenic microorganisms. The mode of action of the LPS relies on the production of short-lived intermediary oxidation products of the thiocyanate ion, principally hypothiocyanite (OSCN-).

As will be noted, the hypothiocyanite ions react with bacterial membranes, as well as impair the function of bacterial metabolic enzymes; hence their antimicrobial effects (Mickelson, 1977; Reiter & Marshall, 1979). Hypothiocyanite ions are short-lived, surviving only approximately 400 minutes after the initiation of the LPS reaction. At the conclusion of treatment with the LPS, only lactoperoxidase, glucose oxidase, glucose, and sucrose remain. Thiocyanate, hydrogen peroxide, and hypothiocyanate are consumed during the process; residual levels are negligible. Due to the short life of the active ingredients, the LPS is a processing aid for use in extending the shelf life of variety of dairy products, specifically fresh cheese including mozzarella and cottage cheeses, frozen dairy desserts, fermented milk, flavored milk drinks, and yogurt.

1.4. Basis for GRAS Determination

Pursuant to §170.30, Taradon Laboratories has determined, through scientific procedures, that its lactoperoxidase system is GRAS for use as a processing aid to extend the shelf life of a variety of dairy products, specifically fresh cheese including mozzarella and cottage cheeses, frozen dairy desserts, fermented milk, flavored milk drinks, and yogurt, at levels not to exceed 300 mg/L milk.

1.5. Availability of Information for FDA Review

The data and information that are the basis for GRAS determination are available for the FDA's review and copies will be sent to FDA upon request. Requests for copies and arrangements for review of materials cited herein may be directed to:

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1111 Pennsylvania Ave, NW
Washington, DC 20004
(202) 739-5610
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2. IDENTITY OF THE NOTIFIED SUBSTANCE

Peroxidases, including lactoperoxidase and glucose oxidase, are enzymes (proteins) that are part of the natural, non-immune defense systems in milk and in secretions of exocrine glands such as saliva, tears or intestinal secretions.
These systems of protection, which are less specific than the elements of the immune system, play a defensive role against the invasion by bacteria of the mucous membranes. Peroxidases do not have any antimicrobial activity of their own, but in the presence of specific substrates they constitute a powerful system of defense.

These substrates are hydrogen peroxide H_2O_2, and, depending on the specificity of the enzyme, thiocyanate (SCN^-),chloride (Cl^-), bromide (Br^-) or iodide (I^-). Different peroxidases have different functions, for example, myeloperoxidase, which is present in the leukocytes, catalyzes the oxidation of Cl^-, Br^-, SCN^-, I^- ions, lactoperoxidase catalyzes the same reactions except for Cl^-, whereas horseradish peroxidase catalyzes the oxidation of I^- only.

The oxidation reaction catalyzed by these well recognized enzymes can be summed up as follows:

$$H_2O_2 + X^- \longrightarrow H_2O + OX^-$$

Where $X^- = SCN^-, Cl^-, Br^-$ or I^-

The oxidation product, OX^-, is a short-lived oxidizing agent which will react, for instance, with NH_2 groups or thiols (-SH) of the enzymes essential to the metabolism of the bacteria.

The product that is the subject of this Generally Recognized as Safe (GRAS) notification is the Lactoperoxidase System (LPS). It is not a single enzyme but a system consisting of 5 components: the lactoperoxidase enzyme, the glucose oxidase enzyme, the sodium thiocyanate, sucrose and glucose. The enzyme lactoperoxidase catalyzes the oxidation of thiocyanate using glucose oxidase as a source of H_2O_2 and generates intermediate products with antibacterial properties. These products have a broad spectrum of antimicrobial effects against bacteria, fungi and viruses (de Wit and van Hooydonk, 1996; Naidu, 2000, Wolfson and Sumner, 1993)

Three of the components of the LPS – namely sucrose, glucose, and glucose oxidase, are GRAS ingredients for use in the foods. There is a specific GRAS Notification (GRN) 89 for Glucose Oxidase. Lactoperoxidase, as part of the Milk Basic Protein, has been reviewed by the FDA in GRN 196, and received a "No Questions" letter in September 2006. As a result, only the sodium thiocyanate has not been the subject of a public GRAS review.

2.1. Chemical Name

Lactoperoxidase system, consisting of the lactoperoxidase enzyme, the glucose oxidase enzyme, the sodium thiocyanate, sucrose, and glucose.

2.2. Formula

300 ppm of a powder which will be used in 1 liter of the dairy products contains:
Lactoperoxidase: 1.25%

Glucose oxidase: 0.75%
Glucose: 30%
Sodium Thiocyanate: 5%
Sucrose: 63%

2.3. Composition

A. LPS

As stated above, the LPS system contains sucrose, glucose, glucose oxidase, lactoperoxidase, and sodium thiocyanate. As glucose oxidase and glucose are well-defined GRAS substances, a detailed discussion of these components will be reserved. Reference is made to GRN 89 for additional information on glucose oxidase, and 21 C.F.R. 184.1857 for additional information on glucose, 21 C.F.R. 184.1854 for information on sucrose, and the safety of their use in foods. Reference is also made to GRN 196 for additional information on lactoperoxidase. It is important to note that the LPS is listed as a processing aid by the Codex Alimentarius (Annex 1).

B. Lactoperoxidase

Lactoperoxidase is the enzyme that occurs most abundant in cow's milk, after xanthine oxidase. Its concentration ranges from 20 to 50 mg per liter of milk. The values given in various works differ owing to the variety of substrates that have been employed to measure the oxidation of hydrogen acceptor (Oram et al., 1966a; Kiermeier, et al., 1972; Gothefors et al., 1975 and Schindler et al., 1976).

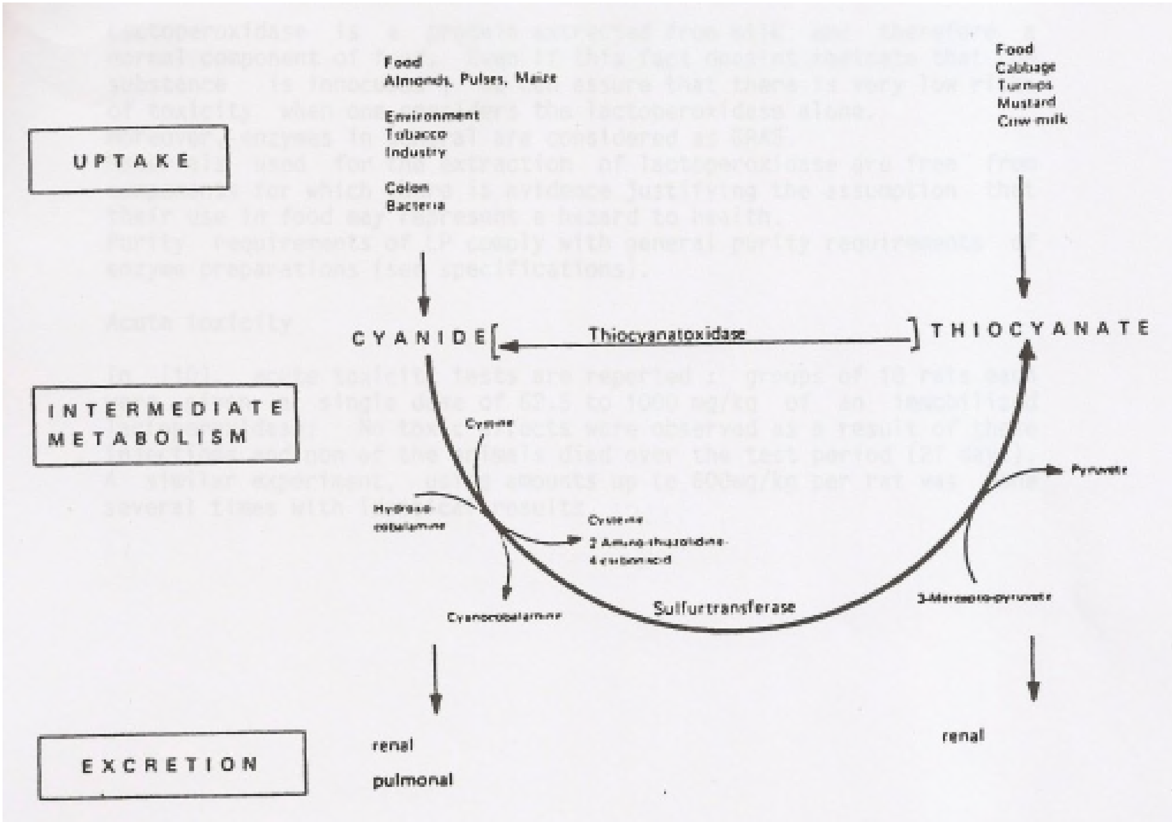
Lactoperoxidase is a single chain glycoprotein containing 612 amino acids and with a molecular weight of about 78,000 daltons (Björck, 1990, 1992; Ekstrand, 1994). Lactoperoxidase is one of the most heat stable enzymes in the milk (Griffith, 1986). Its destruction has been used as an index of pasteurization efficiency of milk. During pasteurization (i.e., heating to 70°C during 15s), whole milk lost three-quarters of its LP activity.

The LP enzyme has no antimicrobial activity by itself. It is the molecule $OSCN^-$ produced by the reaction catalyzed by the LP, which has an antimicrobial activity.

C. Thiocyanate

Thiocyanate (SCN^-) occurs ubiquitously in tissues and secretions of mammals. It is present in the mammary, salivary and thyroid glands and their secretions; in organs such as the stomach and kidney; and in fluids such as synovial, cerebral, cervical and spinal fluids, lymph, and plasma. The concentrations depend partly on the feeding regime of the animal, and eating and smoking habits of man. The source is the anion itself, its esters and other precursors such as nitriles, isothiocyanate, and cyanide. It is produced by the metabolism of sulfur amino acids and the detoxification of cyanide (Figure 1), a well-recognized biological function common to man and animal.

Figure 1: Detoxification of cyanide by the sulfurtransferase thiosulfate



The detoxification of the cyanide in the body is catalyzed by rhodanase (sulfurtransferase thiosulfate) occurring in liver and some bacteria. Cyanide reacts with thiosulfate, a product of sulfur amino acid metabolism, to convert cyanide into thiocyanate (SCN^-).

Plants such as clover contain high concentrations of cyanide and are detoxified in ruminants. Plants contain two main groups of SCN^- precursors: glucosinolates and glucosides. Glucosinolate-rich plants belong to Brassicacae, species of Cruciferae (cabbage, kale, SCN-content up to 600 mg/kg, or 10mM). The hydrolysis of glucosinolate is catalysed by thioglucosidase (myrosinase), producing SCN^- and/or isothiocyanate and nitriles. Glucosides are present in potatoes, maize, millet, sugar cane, peas etc. Hydrolysis of the glucosides in the plants directly yields SCN^- (Michajovskij, 1964; Virtanen, 1961 and Virtanen et al., 1960).

In addition to the above, thiocyanate is naturally present in bovine milk; the normal levels depend on the levels of thiocyanate in animal's diet. Concentrations have been reported to vary between 2.3 and 35 mg/l in milk from individual cows.

The high thiocyanate concentrations in saliva has been generally demonstrated, and at one time saliva was thought to be the only source of SCN^- in human gastric juice. It is now accepted that the parietal cells actively secrete SCN^-. The SCN^- concentration in adults human gastric juice is high, 0.38 mM (22 mg), and even higher than in saliva; up to 2.5 mM SCN^- (145 mg) has been found for the saliva of smokers. Newborn infants have SCN^- anions in their saliva and in their gastric juice, less than that of adults.

The concentration of thiocyanate in the saliva and milk depends partly on the feeding regime of the animal, and eating and smoking habits of man. In case of the smokers, the SCN^- is produced by the metabolism of the sulfur amino acids and the detoxification of cyanide, one of the products of burning tobacco. It has been demonstrated for a long time that the level of SCN^- is influenced by the fodder. Cows grazing natural pastures with a complex flora of different grasses, weeds and clover were shown to give milk with the highest concentrations of SCN^- as between 0.26 mM (15 mg of SCN^- anion) to 0.35 mM (20 mg of SCN^- anion).

As noted above, thiocyanate is present in man, plants, and animals at variable levels. As to LPS use in dairy products, the proposed maximum levels of thiocyanate, the estimated intake of SCN^- for the consumers of LPS-treated dairy products is estimated to be between 15 mg to 20 mg of SCN^- ions per liter of milk. Therefore, the intake of SCN^- anions for an average consumer of LPS-treated dairy products would appear greater than the background from general milk consumption. However, this does not take into account that in the LPS, the SCN^- is converted to innocuous derivatives such as $OSCN^-$ ions, thus reducing the SCN^- levels or to be eliminated by the kidney and the liver. That is why under the actual use conditions as proposed in this notice, the total content of thiocyanate, once the LPS is activated in a mixture, does not surpass the natural maximal concentration in any particular cow milk.

2.4. Specifications for food grade material

The release specifications for the Taradon LPO System are as follows:

Physical/Chemical Specifications:

Property	Average	Minimum	Maximum
Moisture	0.1	0.05	0.2
Fat	0	0	0
Ash	1.5	1.2	1.7
Protein	7.4	6.5	8.5
Density	0.8	0,75	0.85
Refractive Index	NA	NA	NA
Viscosity	NA	NA	NA
Flash Point	NA	NA	NA
Granulation (list pertinent Min. & max. % On/through sieves)	0.1	0.08	0.3

Microbial Specifications:

Type	Count	Sample
Aerobic Plate Count	<50	1g
Coliform	Absent	10g

E. coli*	Absent	10g
Yeast	Absent	1g
Mold	Absent	1g
Coagulase Positive Staphylococcus	Absent	10g
Salmonella	Absent	25g
Other: Listeria species	Absent	25g

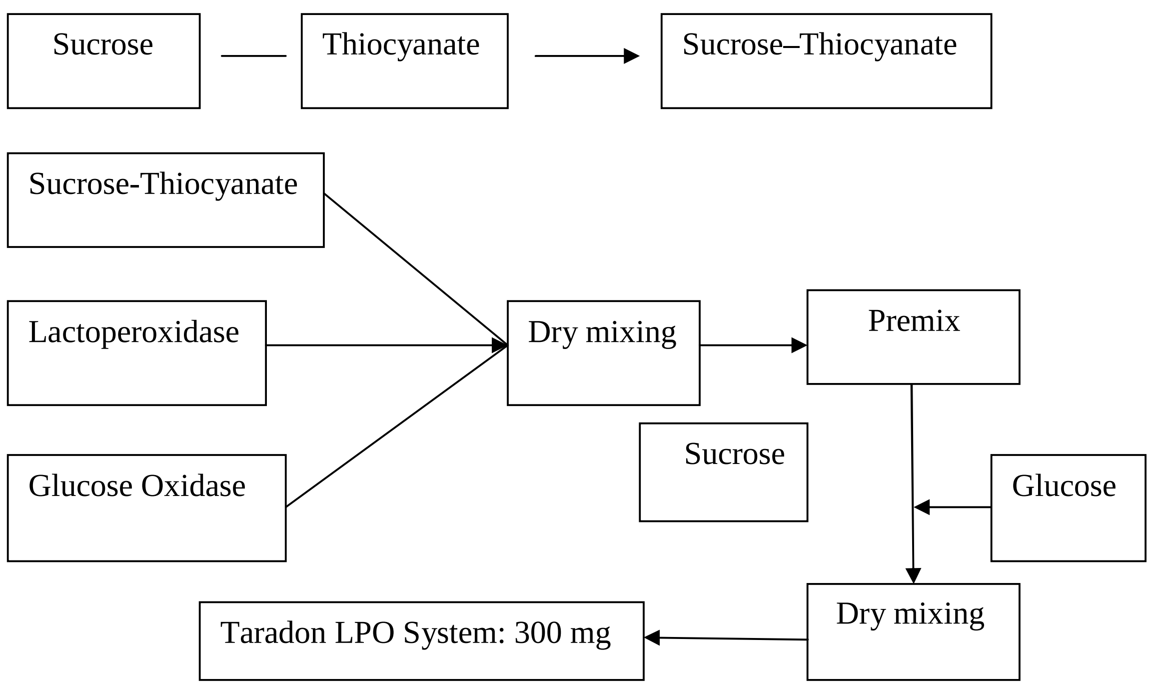
Sensory Specifications:

Property	Standard Description or Target, Minimum, Maximum
Color	Creamy white
Flavor	Sweet
Texture	Dry powder

2.5. Method of Manufacture

A schematic of the production of the Taradon LPO system is shown below. Taradon's production and commercialization of enzymatic preparations has been certified from the British Retail Consortium.

Figure X: Production Scheme



The ingredients of the LPS system can be added to the desired product individually during production, or can be mixed to create the LPS prior to the start of the food production and stabilized with an inert support (such as sucrose), and added in the desired volume, during production.

The product must be kept in a cool, dry place. The shelf-life of the LPS is 3 years unopened, and 1 month following opening, assuming it was stored properly. A certificate of analysis accompanies each shipment, documenting compliance with the release specifications.

2.6. Characteristics and Mechanism of Action

The LPS is considered as a natural defense system against microbial infections. All its components occur naturally in human and animal secretions. The system elicits antimicrobial activity against a wide spectrum of spoilage and pathogenic microorganisms.

The mode of action of the LPS relies on the production of short-lived intermediary oxidation products of the thiocyanate ion, principally the hypothiocyanite ions ($OSCN^-$). The overall reaction is as follows

$$\text{Glucose} + H_2O + O_2 \xrightarrow{\textit{Glucose oxidase}} H_2O_2 + \text{glucuronate}$$

$$H_2O_2 + SCN^- \xrightarrow{\textit{Lactoperoxidase}} HOSCN/OSCN^-$$

These $OSCN^-$ ions in turn reacts with the bacterial cytoplasmic membranes, as well as impair the function of metabolic enzymes, hence their antimicrobial effects (Mickelson, 1977; Reiter and Marshall 1979)

These $OSCN^-$ ions have a short-lived intermediary (+/- 400 minutes) after the starting of the LPS reaction. Due to the short-lived of these ions, the LPS can be considered as processing aids for the production of dairy products.

To understand the reaction mechanism of the lactoperoxidase system, it is important first to determine the structure of the enzyme. Reiter and his collaborators (Oram et al., 1966a; Reiter et al., 1964) showed that an intermediary oxidation product of SCN^- catalyzed by LP and H_2O_2 generated metabolically by the organisms was responsible for the inhibition of some strains of lactic acid streptococci, although some other strains have shown some resistance (Oram et al., 1966b). To understand this mechanism reaction of the lactoperoxidase system, it is important first to understand the structure of the enzyme.

The following four peroxidases, lactoperoxidase, myeloperoxidase, eosinophil peroxidase and thyroid peroxidase constitute the mammalian peroxidases which are distinguished from the peroxidases from plants, fungi and bacteria. Most of the peroxidases, including LP contain ferri-protoporphyrin IX as a prosthetic group (Naidu, 2000; Rae et al., 1998). A characteristic feature of haemoprotein peroxidases is their ability to exist in various oxidation states. There are five known enzyme intermediates for lactoperoxidase. The major intermediates for LP are 1) ferric peroxidase (the native enzyme), 2) Compound I, 3) Compound II, 4) Compound III, and 5) ferrous peroxidase (Pruitt et al., 1991).

The peroxidative reactions are complex and follow different pathways depending upon the concentration of H_2O_2 and whether or not exogenous electron donors are present (de Wit and van Hooydonk, 1996). The first step in the enzymatic mechanism is the initiation reaction of the resting LP (Fe^{3+}) to its ground state, using H_2O_2:

$$Fe^{3+} \quad + \quad H_2O_2 \longrightarrow Fe^{2+} \quad + \quad HO_2^{\cdot}$$

followed by the propagation reactions as illustrated in the figure 5. The superoxidase radical ($HO_2^{\cdot}$) plays an important role in termination of the catalytic reactions to the resting LP (de Wit et al., 1996).

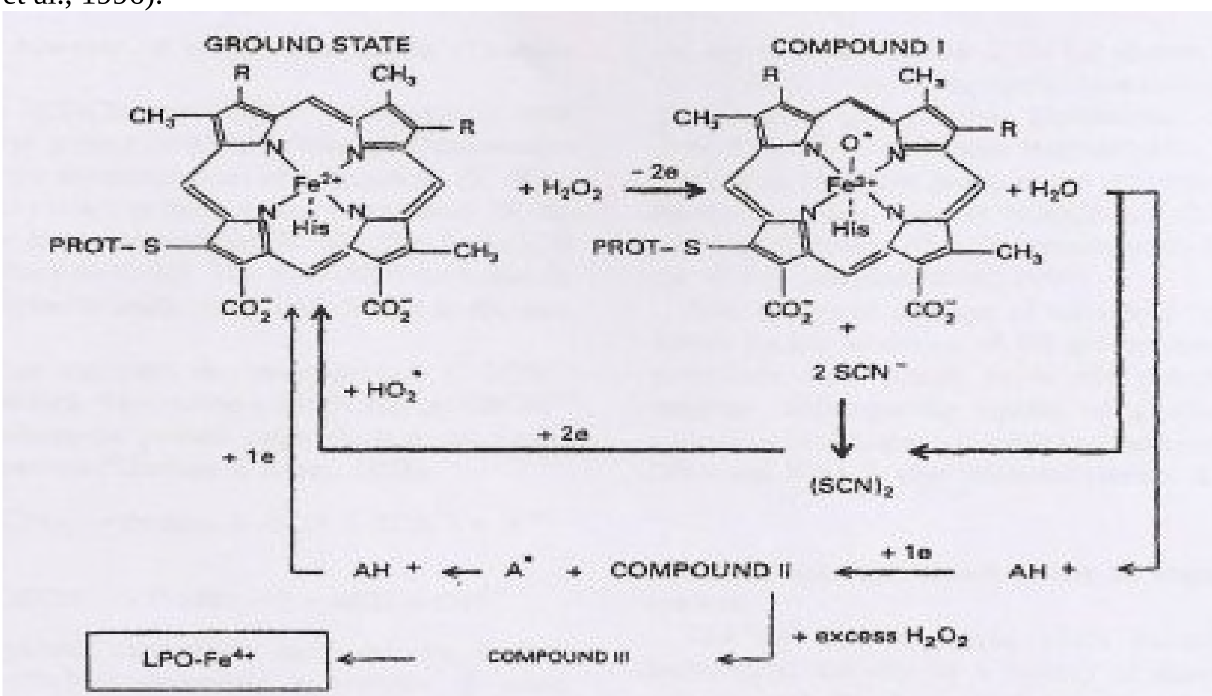


Figure 5: Pathways in the lactoperoxidase-catalyzed reaction mechanism. The normal peroxidalic cycle includes compound I. Insufficient 2-electron donors lead to compound II, and excess of H_2O_2 results in the formation of compound III (de Wit and van Hooydonck, 1996)

The propagation reaction includes the conversion of LP from the ground state into the so-called Compound I state by reaction with H_2O_2. At low SCN^- (<3μM) and halide concentrations. Compound I reacts with H_2O_2 and with any one-electron donor that may be present (such as proteins, peptides, etc.) to form Compound II. Compound II is continuously reduced to the ground state at a low rate. If there is an excess of H_2O_2 (>0.5 mM), Compound II may react with

H_2O_2 to form Compound III, leading to a ferrylperoxidase adduct Compound III is involved in metabolic reactions, leading to irreversible inactivation of LP. The oxidant in peroxidase-catalyzed halogenations is not H_2O_2 itself but rather the reaction product of peroxidase with H_2O_2, known as Compound I (de Wit et al., 1996), that is, the thiocyanate ion (SCN^-) is oxidized by Compound I by a direct two-electron transfer of oxidizing equivalent (Pruitt et al., 1991). The next reaction is:

$$\text{Compound I} + X \longrightarrow \text{Ferric enzyme} + XO$$

Where X represents the halide or the thiocyanate ion and XO is the oxidized product. The products of peroxidation of two-electron donors kill or inhibit the growth and metabolism of many species of microorganisms (Pruitt et al., 1985).

In general peroxidation of H_2O_2 by LP can occur through three different cycles, resulting in divergent antimicrobial activities (de Wit and van Hooydonk., 1996) as follows:

1. In the presence of sufficient oxidizing halide or SCN^- as 2-electron donor for Compound I, giving optimal activation LP.
2. In the presence of insufficient halide or SCN^- of appropriate redox potential, resulting in dominating I^- electron donors and accumulation of Compound II and reversible inactivation of LP.
3. In the presence of an excess of H_2O_2 resulting in the formation of Compound III, associated with irreversible inactivation of LP.

2.7. Antimicrobial Activity

As noted above, the antimicrobial activity can be broad. Thomas (Thomas et al., 1978) established $OSCN^-$ as an oxidizing agent for bacterial sulfhydryls and proteins to sulfenyl thiocyanate and sulfonic acid derivatives (following the mechanism described here below). This oxidation explains the inhibition of respiration in bacteria and inactivation of SH-depending enzymes in glycolysis. At about the same time, Mickelson (1977) came to the conclusion that a modification by the LPS of sulhydryl on the inner membrane made *Streptococcus* agalactiae impermeable to glucose and glycolysis.

Marshall and Reiter have also demonstrated (1980), that OSCN- damages the cytoplasmic membrane by the oxidation of SH-groups in E.coli leading to the leakage of potassium ions, amino acids and polypeptides into the medium. Subsequently uptake of glucose, amino acids, purines, pyrimidines in the cell and the synthesis of proteins, DNA and RNA is also inhibited (Reiter and Härnulv, 1984).

The effect on the cytoplasmic membrane of Gram-positive bacteria by the LP-system has also been demonstrated by the inhibition of amino transport in *Lactobacillus acidophilus* (Clem et al., 1966 and Slowey et al., 1968) and *Staphylococcus aureus* (Hamon et al., 1973) of glucose transport in *Streptococcus agalactiae* (Michelson, 1977) in *E.coli* (Wray et al., 1987) and of oxygen (Reiter and Pickering, 1964). The LP-system inhibits the active transport of glutamic acid, lysine, valine and phentlalanine in *L. acidophilus* (Clem et al., 1966 and Slowey et al., 1968).

Different groups of bacteria show a varying degree of sensitivity to the LP-system. Gram negative, catalase positive organisms such as *Pseudomonas, Coliforms, Salmonella* and *Shigellae,* are not only inhibited by the LP-system but also depending on the medium conditions, may be killed. Gram-positive, catalase negative bacteria, such as *Streptococci* and *Lactobacilli* are generally inhibited but not killed by the LP-system (Oram and Reiter, 1966). This difference in sensitivity can be explained by the difference in cell wall structure and their different barrier properties (de Wit and van Hooijdonk, 1996). The inner membrane of Gram-negative bacteria appears to be more extensively damaged by LP-treatment than with Gram-positive species (Marshall and Reiter, 1980)

The $OSCN^-$ ions are bactericidal for enteric pathogens including multiple antibiotic resistant *E. coli* strains (Naidu, 2000). The $OSCN^-$ ions damages the inner membrane causing leakage and cessation of uptake of nutrient. The antimicrobial activity of the LP-system against *E. coli seems to be related to the oxidation of bacterial sulfhydryls (Thomas and Aune, 1978).* The oxidation of sulphydryls to sulphenyl derivates inhibit the bacterial respiration, but another groups of researchers have identified that the inhibitory effect was due to the inhibition of the dehydrogenases in the respiratory chain of *E. coli*.

The issue of whether long-term use of the LPS would result in any microbiological risks, e.g. development of LPS-resistant strains, antibiotic resistant or toxin-producing bacteria was considered by the FAO/WHO technical committee (2005). The committee concluded that that the available data indicate that adoption of the LPS is not likely to stimulate the development of resistance to the LPS itself or antibiotic-resistant microorganisms (Annex 2). This report is discussed in further detail in section 3.2.

2.8. Potential Toxicants

A reaction product of the LPS system is hydrogen peroxide, or H_2O_2. As noted above, H_2O_2 is a critical component of the system, and rather than adding H_2O_2 directly, it is instead formed in the reaction of glucose, oxygen, and water.

$$H_2O + \text{glucose} + O_2 \longrightarrow \text{gluconic acid} + H_2O_2$$

Although hydrogen peroxide is generated by the oxidation of glucose that occurs naturally during the action of glucose oxidase, it is generally assumed to be not present in milk or dairy products. This is because H2O2 is rapidly reduced during the enzymatic oxidation of thiocyanate to produce the hypothiocyanite ion, producing water. In bovine milk, the production of $OSCN^-$ catalyzed by lactoperoxidase depends on the levels of SCN^- and H_2O_2. In the past, International Dairy Federation (IDF) has recommended the use of 300-800 ppm. H_2O_2 for the preservation of milk wherever adequate cooling is difficult, as in developing countries. Since such excessive concentrations affect the clotting of milk inactivate enzymes and denature proteins through the oxidation of amino acids (tryptophan, tyrosine, methionine, histidine and cystine (Methods of Enzymology; vol XI, 3rd Edit. N.Y. Acad Press) the residual H_2O_2 should be eliminated by heat treatment and addition of catalase - a rather complex procedure. Treatment of milk by the LPS requires only very low levels of H_2O_2-10-15 ppm sufficient to oxidize SCN^- in the presence of lactoperoxidase and without affecting the enzyme. Further, these levels are below the levels permitted for use in dairy products for cheese making, as noted in 21 C.F.R. §184.1366

It is interesting to note that at any moment hydrogen peroxide is consumed by the lactoperoxidase/thiocyanate system and that it would never exceed 10 µM: i.e. 3 % of the dose recommended by the International Dairy Federation for the preservation of raw milk by activation of the LPS (Annex 3). The toxicology of H_2O_2 has been reviewed in the Department of Health and Family services in 1993, has also been evaluated in an IARC monograph in 1985 and by ECETOC (Joint Assessment of Commodity Chemicals N° 22, January, 1993). The US Environmental Protection Agency, after a full toxicological assessment, has established an exemption from the requirement of a tolerance for residues of the biochemical H_2O_2 on all food commodities when used as an algaecide, fungicide and bactericide at the rate of 1% H_2O_2 per application on growing crops and post-harvest crops (vol 64, N° 118, June 1999). Exogenous H_2O_2 decomposes to oxygen and water on contact with tissues, thus limiting absorption of the intact molecule.
Any H_2O_2 molecule produced is immediately used by the lactoperoxidase so that peroxide cannot accumulate in solution (Reiter et al., 1976). Therefore, there is no concern of any potential toxicity with H_2O_2.

3. BASIS FOR GRAS DETERMINATION

3.1. History of Safe Use

The LPS has been approved as a processing aid to extend the shelf life of dairy products by various international regulatory and scientific advisory bodies including: Codex, the Joint FAO/WHO Expert Committee on Food Additives (JECFA), Food Standards Australia New Zealand (FSANZ), the French Agency for Food Safety (AFSSA), and others.

In a well written and concise document, the Codex document Codex Code of Practice, Guidelines for the Preservation of Raw Milk by Use of the Lactoperoxidase System, CAC/GL 13-1991 sets forth the Codex-approved specifications and practices for use of the LPS for the stabilization of milk (Codex Committee on Milk and Milk Products, 2012). Codex notes that refrigeration remains the method of choice for safe milk transport. The Codex-approved LPS utilizes the lactoperoxidase already present in milk and the system is initiated by sodium percarbonate (rather than glucose and glucose oxidase) to generate the hydrogen peroxide necessary to convert thiocyanate to hypothiocyanite.

In Sweden, the National Food Administration has evaluated the efficiency of the LPS and existing toxicological data and has decided to allow the use of LP-activation in milk where raw milk cannot be properly cooled (The National Food Administration, 1980) (Sweden, 1980; Swedish Waterhouse, 2012).

The LPS was approved by the National Expert Committee on Food Additives in the People's Republic of China as "an acceptable preservative used for milk preservation."

In France, the Ministry of the Economy of Finance and Industry gave a permit for the addition of the LPS to the brine "destined for the production of smoked salmon" in April 1998. In 2003, the AFSSA (French Food Safety Agency) authorized the use of the $OSCN^-$ ions (oxidation product of the SCN^-) without the presence of the LPS, as a processing aid for the treatment of fresh-cut, ready-to-eat salads (Agence Française de Sécurité Sanitaire des Aliments (AFSSA), 2012). In 2002, the Finnish Ministry approved the system for similar uses.

In Australia and New Zealand (2002), the FSANZ approved the use of the LPS containing 40 mg/liter of SCN- in the agro-food industry as a processing aid functioning as an antibacterial agent for meat and meat products.

In 1990, JECFA concluded that the LPS was acceptable for use in milk preservation and does not present a toxicological hazard (FAO/WHO, 2005; JECFA, 1990; JECFA, 2005). In 2005, an FAO/WHO technical meeting concluded that the LPS is "a safe method of preventing milk losses due to microbial spoilage when used according to the Codex guidelines either alone or in combination with other approved procedures."

These uses demonstrate the safe use of the LPS in dairy products.

3.2. Summary of Literature

3.2.1. FAO/WHO Technical Report

In 2005, the FAO/WHO Technical Meeting was held to evaluate the use of the lactoperoxidase system for preservation of raw milk. The resultant report of that meeting discusses in detail the LPS, as well as the potential risks and benefits of its use. This report is included in Annex 2.

The report discusses the efficacy of the LPS, and acknowledges its broad antimicrobial activity against bacteria, viruses, mold, yeasts, protozoa, and other milk spoilage microorganisms. The mechanism of action is considered primarily bacteriostatic, and also points out that the LPS does not promote microbial growth or encourage resistance. Further, the report also clearly states that use of the LPS cannot be used to disguise or hide spoiled milk.

FAO/WHO Technical Group devoted a significant portion of the report to the safety of the LPS, and the report includes an extensive review of the literature pertaining to the use of the LPS and thiocyanate. The authors affirm that hypothiocyanate is found in saliva, and has a short half-life in milk, making the residual levels of no concern of safety. The report also discusses the extensive list of studies performed in iodine deficient populations and those with thyroid disorders, given the potential concern for interference with iodine metabolism at very high plasma levels of thiocyanate. While there is some evidence of mild alterations after consumptions of 45 milligrams, levels which are much higher than intended for the used proposed in this GRAS notice, other studies found no alternation in thyroid function, even in iodine deficient populations. They also evaluated a study conducted over a 10 year period in the American tropics, with no adverse effects of LPS treated milk found. Reference is made to a 2-year rat carcinogenicity study of sodium thiocyanate, which found no evidence of carcinogenicity. The Technical Group concluded that there was no significant toxicological risk to the general public from consumption of LPS.

The report concluded that the LPS is a safe and effective method for preservation of raw milk. The FAO/WHO Technical Group believed that the system had numerous advantages, and no significant risk that would prevent its application to the global community.

3.2.2. Other Relevant Scientific Articles

While the FAO/WHO report provides a comprehensive review of the relevant literature, we also wished to highlight several studies which also demonstrate both the safety and effectiveness of the LPS and thiocyanate in dairy products. The referenced scientific articles are provided in Annex 4.

As noted in several locations in this document, the presence of lactoperoxidase and thiocyanate has been well documented in human and infant saliva. As shown in a paper published in 1975, the levels of lactoperoxidase and thiocyanate present in infant saliva, though one third of the level present in adults, is still sufficient to exhibit antimicrobial activity (Gothefors and Marklund, 1975). Interestingly, the levels of lactoperoxidase vary, with some levels higher than those seen in adults observed. The authors also conclude that the presence of the lactoperoxidase activity is present in both humans and cows, underscoring its biological significance, as well as its prevalence. The use of the LPS under the conditions proposed in this notification would not be an introduction to a new substance in the human population.

Another paper published in 1975 explores the efficacy of the LPS against milk spoilage organisms (Bjorck, et al., 1975). The authors determined that the LPS was antimicrobial against several gram-negative bacteria including certain strains of *E.coli* and *Pseudomonas*. The importance of glucose and glucose oxidase was elucidated, and was found to be a key component of the system, supporting the production of hydrogen peroxide. The paper also notes that the system is removable, and has no lasting impact on the milk once the LPS components have been removed.

Two studies have also evaluated the efficacy of the LPS against *Listeria monocytogenes*. The first used a model broth culture system, and found that against the strain Scott A, LPS exerted a bacteriostatic effect, rather than a bactericidal effect (Siragusa and Johnson, 1989). However a second study which evaluated multiple strains in raw milk found the LPS has a bactericidal effect, but this effect is strain and temperature dependent (Gaya, Medina, and Nunez, 1991). The LPS in this study against *Listeria monocytogenes* was most effective at refrigeration temperatures. Given that the intended use of the product in this notification is the prolongation of shelf-life, the ability to prevent the growth of bacteria also important. Further, as has been stated numerous times, the use of the LPS does not negate the need for pasteurization, and also assumes users will use the appropriate manufacturing and processing techniques to ensure a safe end product.

A review article published in 2005 contains an extensive discussion on the mechanism of action of the LPS, as well as an overview of the efficacy studies performed with the LPS (Seifu, Buys, and Donkin, 2005). These studies have found a bactericidal effect on numerous gram-negative bacteria, including *H. pylori*, *Actinobacillys actinomycetemcomitans*, and *Fusobacterium nucleatum*, and a gram positive bacteria *Streptococcus sanguis*. Growth and or enzyme inhibition were also noted for a variety of bacterial strains, including *Streptococcus mutans,* and *Yersinia enterocolitica*, as well as the HIV-1 virus. The LPS was also bactericidal and bacteristatic against Staph aureus, a major cause of bovine infections as well as human infections. The LPS was also found to be bactericidal against the human pathogens *Salmonella typhimurium* and *Campylobacter jejuni*, as referenced by the authors. The specific mechanism of action is dependent on the type of pathogen the LPS faces, and multiple mechanisms are reviewed. Another review, published by Taradon Laboratories in conjunction with Liege University Plant Pathology Laboratory, provides an extensive review of the chemical actions of the LPS, specifically focusing on the antimicrobial activity (Bafort, et al., 2014). Both of these review articles support the efficacy of the LPS for the intended use, as a processing aid to extend the shelf-life of certain dairy products.

Finally, a study directly relating to the proposed use of this notification, an extension of shelf life, was conducted in 2015 (Pokhrel and Das, 2012). This study evaluated the ability of the LSP to extend the shelf life of raw milk. The LPS provided a significant increase in shelf life compared to control at temperatures of 25°C and 5°C. In the 5°C group, shelf life of milk was extended by 2 days. The paper underscores the efficacy of the LPS for the intended use.

3.3. Toxicology Studies

Evaluation of the toxic risks connected to the utilization of all components of the Lactoperoxidase System (LPS) from which we require a GRAS status, implies to evaluate the toxicity of each particular ingredient (i.e. lactoperoxidase, thiocyanate, glucose oxidase, sucrose and glucose as it is described previously in this document) as well as the toxicity of the oxidation products of thiocyanate formed during the reaction. Considering the fact that glucose, glucose oxidase (GRN 89) and lactoperoxidase (under its evaluation GRN 00196), only the thiocyanate (SCN^-) requires a safety evaluation.

As explained previously, the antibacterial effect of the LPS is mediated by short-lived oxidation products of thiocyanate. These intermediates are very unstable and those not reacting with bacteria decompose spontaneously. Products treated with LPS would not have any active agents when they reach the consumer.

In the case of the use of the glucose/glucose oxidase and in absence of microorganisms, the end products of the reaction are SO_4^{--} , CO_2, NH_4^+ and gluconic acid and water. These products are not toxic.

If from a theoretical point of view, thiocyanate can be regenerated from the reduction of hypothiocyanite (i.e. when $OSCN^-$ reacts with bacteria), we have failed to show this effect *in vitro.*

Toxicological risks associated with the addition of SCN^- to foodstuffs at the proposed levels of use would be very low, because we can assume that all the SCN^- is consumed by the system, and the toxicology studies conducted to date support this conclusion.

Below are the summaries of the safety studies performed to date. Some of these studies have been published and others are unpublished studies.

A. Acute Toxicity

Acute toxicity of the LPS was tested in mice and rats at two dosage levels, one optimized to produce the highest levels of hypothiocyanite and one which delivered all four ingredients up to their solubility limit. The latter formulation produces no hypothiocyanite because of the excess hydrogen peroxide present.

The LPS was administered orally in water (25mL/kg) to "Souris" OF1 mice, 10M and 10F/group) after an 18-hr fast. The mice were observed for 15 days. There was no control group. The Lp-system Formula A (maximal hypothiocyanite) contained 4,000 mg/L glucose, 18.72 mg/L lactoperoxidase, 2 mg/L glucose oxidase, and 68.9 mg/L sodium thiocyanate; the total dose was 102 mg/kg bw). The LPS Formula B (maximum dose) contained 625 g/L glucose, 2.9 g/L lactoperoxidase, 0.32 g/L glucose oxidase, and 10.7 g/L sodium thiocyanate; the total dose was 16 g/kg bw. Formula B is approximately 165 times higher than that delivered in Formula A.

There were no deaths, signs or toxicity or abnormal weight gain in the mice receiving Formula A. Necropsy revealed no lesions other than desquamation of the stomach mucosa (10/10 males and 1/10 females) and red spots on the mucosa of one male.

Four of ten male mice died and no female mice died after receiving Formula B. No toxic symptoms were observed in the female mice. Sedation was observed in two of the surviving males and return reflex was inhibited in one of the surviving males. Weight gain was transiently lower at day 5, but returned to normal by day 10. Necropsy revealed bleeding (1/10 M) and desquamation of stomach mucosa (7/10 F). No other signs of toxicity were observed. The authors conclude the LD0 for males was greater than 102 mg/kg bw but less than 16 g/kg bw and for females the LD0 was greater than 16 g/kg bw. It is important to note that Formula B is far beyond any dose that would be administered in the proposed levels of use.

The LPS was administered orally in water (10 mL/kg) to Sprague Dawley OFA rats (10M and 10F/group) after an 18-hr fast. The rats were observed for 15 days. The Lp-system Formula A (maximal hypothiocyanite) contained 4,000 mg/L glucose, 18.72 mg/L lactoperoxidase, 2 mg/L glucose oxidase, and 68.9 mg/L sodium thiocyanate; the total dose was 40.9 mg/kg bw). The Lp-system Formula B (maximum dose) contained 833 g/L glucose, 3.9 g/L lactoperoxidase, 0.42 g/L glucose oxidase, and 14.3 gm/L sodium thiocyanate; the total dose was 8.5 g/kg bw. No control group was included. No deaths, signs of toxicity, or abnormal weight gains were observed for either the Formula A or Formula B groups. The authors conclude that the LD0 is greater than 8.5 g/kg bw.

Cannulated calves (Reiter et al., 1980) were fed 200 ml of raw milk containing *E.coli* , followed by 2000 ml of raw milk containing lactoperoxidase, thiocyanate, and one of various sources of hydrogen peroxide (either glucose oxidase/glucose or magnesium peroxide or a hydrogen peroxide producing strain of *Lactobacillus casei*). In abomasal samples taken immediately after feeding and periodically thereafter initial inoculums were reduced by at least 99.9%. No adverse effects were reported.

B. Subacute Toxicity

Wang Peng et al. fed dogs with milk supplemented with hydrogen peroxide and thiocyanate (36.95 mg/kg bw/day) for 14 days and observed normal health and weight gain. In another study, mice were treated with milk supplemented with hydrogen peroxide and thiocyanate at 17.8 mg/kg bw/day for 14 days, followed by 59.6 mg/kg bw/day thiocyanate for 11 days, and then 79.7 thiocyanate mg/kg bw/day for 11 days. Rats were treated with 2.71 mg/kg bw/day for 104 days or followed by 34.3 mg/kg bw/day for 9 days, then 51.1 mg/kg bw/day for 70 days, and finally, 57.3 mg/kg bw/day for 25 days. Normal health and weight gain was reported for all experimental groups of rats and mice. No differences from placebo control in blood serum, general appearance, color, consistency, size and weight of liver, kidney, heart, and spleen were reported. Insufficient experimental details are available to permit evaluation of this study.

Reiter et al. (1981) fed neonatal calves (> 200 animals) with either whole milk or milk substitute, both containing LPS (whole milk + 20 ml of a solution containing 1.6 g KSCN/L, 300 g glucose/L, and 20 ml of a solution containing 0.5 g glucose oxidase/L) for 5 weeks or until weaning. Weight gain was increased compared to controls by 3 weeks and sustained until the conclusion of the study. No adverse effects were reported.

Similar results were reported by Still et al. (1990) using young calves fed with a formulation containing the LPS, which is a whey-based feed complement containing lactoferrin and the lactoperoxidase system (20 mg/L lactoperoxidase, 1 mg/L glucose oxidase, 25 mg/L thiocyanate, and 1 g/L glucose). The results showed that LPS significantly increased the weight gain of calves that received this formulation.

These results demonstrate that the lactoperoxidase system can be activated *in vivo* without any adverse effect.

The LPS was administered orally in aqueous suspensions of carboxymethylcellulose (10 mL/kg) to Sprague Dawley OFA rats (10M and 10F/group) daily for 14 weeks. Control (4,000 mg/L glucose) plus three dose levels of LPS: Group B (4,000 mg/kg bw glucose, 0.002 mg/kg bw glucose oxidase, 0.025 mg/kg bw lactoperoxidase, and 0.05 mg/kg bw thiocyanate); Group C (4,000 mg/kg bw glucose, 0.006 mg/kg bw glucose oxidase, 0.075 mg/kg bw lactoperoxidase, and 0.15 mg/kg bw thiocyanate); and Group D (4,000 mg/kg bw glucose, 0.02 mg/kg bw glucose oxidase, 0.25 mg/kg bw lactoperoxidase, and 0.5 mg/kg bw thiocyanate). None of the animals died, no abnormal behaviors were observed, and no adverse effects were noticed during daily clinical examination. Parameters evaluated include: weight evolution, body weight gain, feed consumption, water consumption, ophthalmological examination, hematological examinations, biochemical examination of blood and urine, anatomical examinations, organ weight and histopathological examinations.

In summary, in this study, the lactoperoxidase system has been tested in female and male rats for subacute toxicity during 14 weeks. The experimental protocols have been to be adapted to the specific nature of the LPS and rats were administered solutions containing optimal amounts of SCN- oxidation products.

C. Chronic Toxicity

A two-year chronic toxicity/carcinogenicity bioassay of sodium thiocyanate (alone or in combination with sodium nitrite) has been conducted in F344 rats. The animals received sodium thiocyanate at a level of 3.2 grams/liter in drinking water. The results of this study led to the conclusion that sodium thiocyanate is not carcinogenic to rats (Lijinsky and Kovatch, 1989).

D. Mutagenicity/Genotoxicity Studies

Hypothiocyanite produced by the LPS using hydrogen peroxide, lactoperoxidase, and potassium thiocyanate was found to be cytogenic, but not mutagenic, in the Ames assay using *Salmonella typhimurium* indicator strains TA 1535, TA 1537, TA 1538 and hisG-46. Hypothiocyanite generated enzymatically at an estimated initial concentration of 970 μM and by direct addition of hypothiocyanite at concentrations of 0, 0.11, 0.33, 1.1, 3.3, 11, 33, and 90 μM. Cell toxicity was noted at concentrations of 33 and 90 μM in all four strains. Hypothiocyanite was not toxic for *Saccharomyces cerevisiae* D-7 at concentrations up to 860 μM and did not oxidize calf thymus DNA after *in vitro* incubation for 30 min at room temperature (White, Jr. et al., 1983).

E. Cytotoxicity Studies

The cytotoxic effects of various components of the LPS have been studied alone or in combination for cytotoxic effects. Lactoperoxidase was reported to lyse erythrocytes *in vitro* in the presence of hydrogen peroxide and iodine (McFaul et al., 1986). The cytolysis required the presence of iodine ions and was not observed when iodine was replaced by bromide, thiocyanate, or fluoride.

Moreover, Everse and collaborators (1985) have shown that the peroxidase system has a no toxicity level for normal tissues, but a specific antitumoral action by studying the effect of injection of a mixture of glucose oxidase and horseradish peroxidase immobilized onto small solid beads.

Tenovuo et al. (1984) reported that lactoperoxidase alone (5 ppm), thiocyanate alone (10 mM), or the combination of the two has no apparent effect on ^{3}H-thymidine incorporation, nor did they cause visual damage to the cells in human fibroblasts *in vitro*. Hydrogen peroxide at concentrations of 100 µM caused over 80% reduction in ^{3}H-thymidine incorporation compared to the controls. 200 µM of H_2O_2 was totally inhibitory. Peroxide-treated cells were partially or totally lysed when examined under microscope. Hypothiocyanite generated before addition to the cells at concentrations up to 300 µM had no effect on ^{3}H-thymidine incorporation in this study. Hypothiocyanite generated in presence of the cells by adding varying concentrations of hydrogen peroxide to the medium already containing cells, lactoperoxidase, and thiocyanate had no apparent effect on ^{3}H-thymidine incorporation, as long as there was no unreacted hydrogen peroxide left in the medium.

This study indicates that elevated concentrations of hypothiocyanite at levels that inhibit bacterial metabolism did not damage human cells.

3.4. Estimated Dietary Intake

As noted previously, the components of the LPS system, including glucose, sucrose, and glucose oxidase, have previously been established as GRAS for use in food. Therefore, the component which requires and assessment of safety is thiocyanate. As such, the focus of the exposure assessment was the exposure to thiocyanate. It is important to point out that a significant portion of the thiocyanate is converted into unstable intermediates that decompose spontaneously before consumption. In this estimated daily intake study, this phenomenon is not taken into account, and as a consequence, the exposure study of thiocyanate can be considered as the worst case scenario.

Thiocyanate is proposed for use in the following five milk-based food and beverage categories: fresh cheeses (including mozzarella and cottage cheese), frozen dairy desserts, fermented milk, flavored milk drinks, and yogurt. Table 1 lists the proposed food use categories and their corresponding thiocyanate concentration that is naturally occurring, proposed for use in food, and the total maximum thiocyanate levels in proposed foods which accounts for both the naturally occurring thiocyanate levels in food plus the proposed use levels.

Table 1. Proposed Uses

	Thiocyanate (mg/kg)		
Food Category	Naturally Occurring	Proposed Use	Total (Natural + Proposed Use)
Fresh Cheese			
Mozzarella	15	0*	15
Cottage Cheese	15	15	30
Frozen Dairy Desserts	3	1.5	4.5
Fermented Milk	15	15	30
Flavored Milk Drinks	15	15	30
Yogurt	30	15	45

*The proposed use for mozzarella is in the water the cheese is stored in, not the actual cheese itself.

Using the What We Eat in America (WWEIA) dietary component of the National Health and Nutrition Examination Surveys (NHANES) 2009-2012. consumption data, Exponent estimated the 2-day average daily intake on a *per capita* and *per user* basis. In the analysis, the 2-day average intake of thiocyanate was estimated by multiplying the reported intake of foods from the 24-hr recall with the proposed corresponding thiocyanate use level (see Table 1) and the cumulative sum over the two 24-hr recalls was divided by two. This was then repeated using the maximum levels of thiocyanate (i.e., naturally occurring level plus proposed use level). Intake estimates of thiocyanate were derived from all proposed uses combined for the total U.S. population and expressed in units of milligram per day (mg/day), and are presented below in Table 2. The results are presented in the table below, and the full Exponent report is available in Annex 5. The total use for the mean and 90th percentile users are well below those values established in the toxicology studies.

Table 2. Estimated Exposure

	Total U.S. Population					
			Per Capita (mg/day)		Per User (mg/day)	
EDI based on:	Unweighted N	% User	Mean	90th Percentile	Mean	90th Percentile
Proposed Use	7,576	49	0.59	2	1.2	3.33
Maximum Use (Natural + Proposed)	10,208	67	1.63	5.52	2.44	7.24

The levels from the proposed uses are below the naturally occurring levels found in milk, which range from 2.3 and 35 mg/l in milk from individual cows.

3.5. GRAS Conclusion

The information submitted as part of this GRAS notice demonstrates that the five components of the LPS, lactoperoxidase, glycose oxidase, glucose, sodium thiocyanate, and sucrose are GRAS, which in turn makes the lactoperoxidase system GRAS. Three of the ingredients are present in the human and animal body, including thiocyanate which is present in human saliva and gastric juice. The chemical reaction of the LPS lasts approximately 400 minutes, which would be completed prior to the consumption of the product.

Taradon Laboratories has concluded that the information submitted and referenced allows them to state that the LPS is generally recognized as safe as a processing aid in fresh cheese including mozzarella and cottage cheeses, frozen dairy desserts, fermented milk, flavored milk drinks, and yogurt.

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5. LIST OF ANNEXES

Annex 1 - Codex Alimentarius Listing of the LPS System

Annex 2 - FAO/WHO Technical Committee (2005), Benefits and Potential Risks of the Lactoperoxidase system of Raw Milk Preservation, WHO, Geneva.

Annex 3 - International Dairy Federation Statement

Annex 4 – Selected Literature References

Annex 5 - Exponent Report

Annex 1

Pages 000035-000196 have been removed in accordance with copyright laws. The removed reference citations are:

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Annex 5

Center for Chemical Regulation and Food Safety

D R A F T

Estimated Daily Intake of Thiocyanate

D R A F T
Estimated Daily Intake of Thiocyanate

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Washington, DC 20036

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List of Acronyms

DHHS	U.S. Department of Health and Human Services
EDI	Estimated Daily Intake
FARE®	Foods and Residues Evaluation Program
FDA	U.S. Food and Drug Administration
FNDDS	Food and Nutrient Database for Dietary Studies
FPED	Food Patterns Equivalents Database
NCHS	National Center for Health Statistics
NHANES	National Health and Nutrition Examination Survey
WWEIA	What We Eat In America
U.S.	United States
USDA	U.S. Department of Agriculture

Introduction

At the request of Morgan, Lewis & Bockius LLP (Morgan, Lewis & Bockius), Exponent, Inc. (Exponent) conducted an intake assessment to estimate the total daily intake of thiocyanate proposed for use in the following five milk-based foods and beverages: fresh cheese including mozzarella and cottage cheeses, frozen dairy desserts, fermented milk, flavored milk drinks, and yogurt. The estimated daily intake (EDI) of thiocyanate was based on food consumption data from the 2009-2012 National Health and Examination Survey (NHANES) and provided for the total U.S. population. The data and methods used to conduct the intake assessment and results are summarized in this report.

Data and Methods

Proposed Use and Levels

Thiocyanate is proposed for use in the following five milk-based food and beverage categories: fresh cheeses (including mozzarella and cottage cheese), frozen dairy desserts, fermented milk, flavored milk drinks, and yogurt. Table 1 lists the proposed food use categories and their corresponding thiocyanate concentration that is naturally occurring, proposed for use in food, and the total maximum thiocyanate levels in proposed foods which accounts for both the naturally occurring thiocyanate levels in food plus the proposed use levels. The data on thiocyanate levels in the proposed food categories were provided by Morgan, Lewis & Bockius.

Table 1. Proposed Food Uses and Levels[1]

	Thiocyanate (mg/kg)		
Food Category	**Naturally Occurring**	**Proposed Use**	**Total Maximum (Naturally Occurring + Proposed Use)**
Fresh cheese			
Mozzarella	15	0	15
Cottage cheese	15	15	30
Frozen dairy desserts	3	1.5	4.5
Fermented milk	15	15	30
Flavored milk drinks	15	15	30
Yogurt	30	15	45

[1] Data provided by Morgan, Lewis & Bockius

Consumption Data

Thiocyanate intakes from proposed uses in food were derived using the What We Eat in America (WWEIA) dietary component of the National Health and Nutrition Examination Surveys (NHANES) 2009-2012. This continuous survey is a complex multistage probability sample designed to be representative of the civilian U.S. population (NCHS 2014, 2013). The NHANES datasets provide nationally representative nutrition and health data and prevalence estimates for nutrition and health status measures in the U.S. To produce reliable statistics, NHANES over-samples adults 60 years of age and older, African Americans and Hispanics. Statistical weights are provided by the National Center for Health Statistics (NCHS) for the surveys to adjust for the differential probabilities of selection. As part of the examination, trained dietary interviewers collect detailed information on all foods and beverages consumed by respondents in the previous 24-hour time period (midnight to midnight). A second dietary recall is administered by telephone three to ten days after the first dietary interview, but not on the same day of the week as the first interview. The dietary component of the survey is conducted as a partnership between the U.S. Department of Agriculture (USDA) and the U.S. Department of Health and Human Services (DHHS). DHHS is responsible for the sample design and data collection, and USDA is responsible for the survey's dietary data collection methodology, maintenance of the databases used to code and process the data, and data review and processing. A total of 16,011 individuals in the survey period 2009-2012 provided two complete days of dietary recalls.

Analysis

Using the WWEIA NHANES consumption data, Exponent estimated the 2-day average daily intake on a *per capita* and *per user* basis. In this analysis, a *user* is anyone who reported consuming any of the proposed foods on either of the survey days. We identify each participant who reported consuming a proposed food on either of the survey days, and we use that individual's responses for both survey days. Zero consumption days are included in calculating that individual's average daily intake. For example, if someone reported consuming 100 grams (g) of yogurt on day 1 and 225 g of yogurt on day 2, his/her 2-day average yogurt consumption would be 162.5 g ([100 + 225]/2). The analysis was limited to individuals who provided two complete and reliable dietary recalls as determined by NCHS. The 2-day average intakes by each individual were estimated using Exponent's Foods and Residues Evaluation Program (FARE® version 11.14) software. Exponent uses the statistically weighted values from the survey in its analyses. The statistical weights compensate for variable probabilities of selection, adjusted for non-response, and provide intake estimates that are representative of the U.S. population.

In the analysis, the 2-day average intake of thiocyanate was estimated by multiplying the reported intake of foods from the 24-hr recall with the proposed corresponding thiocyanate use level (see Table 1) and the cumulative sum over the two 24-hr recalls was divided by two. This was then repeated using the maximum levels of thiocyanate (i.e., naturally occurring level plus proposed use level). Intake estimates of thiocyanate were derived from all proposed uses combined for the total U.S. population and expressed in units of milligram per day (mg/day).

Consumption data in the NHANES survey are reported on an "as consumed basis". That is, if a survey participant consumed a roast beef sandwich, the consumption amount reported in the survey for that subject would be for the total amount of the whole sandwich consumed, and not for the ingredients (bread, meat, lettuce, tomato, and mayonnaise) used to make that sandwich. Exponent identified foods reported consumed in NHANES with proposed uses of thiocyanate (see Table 1). The list of NHANES codes (and their description) that was captured in determining the foods with thiocyanate proposed uses are provided in Appendix I. Baby foods were excluded from the analysis.

When only a component of a food consumed was proposed for thiocyanate use, Exponent utilized USDA's Food and Nutrient Database for Dietary Studies (FNDDS), version 2011-2012 (USDA, 2014), which translates the food as consumed into its corresponding ingredients (and gram amounts) or recipes. For example, USDA recipes were used to identify the cottage cheese component in gelatin desserts mixtures and the yogurt component in a gyro sandwich and curry meat dishes. Thus, for foods containing an ingredient that is proposed for thiocyanate use, only the proportion corresponding to that ingredient was captured in the analysis.

In several cases, the USDA recipes did not have a complete breakdown of ingredients and an alternate approach was taken to identify food components with thiocyanate proposed use. A summary of the alternate approaches taken are presented below:

- <u>Mozzarella:</u>
    - Pizza: The amount of mozzarella cheese per 100 grams of food in pizzas was determined based on the Food Patterns Equivalents Database (FPED) 2011-2012

and 2009-2010 (Bowman et al. 2015, 2014). The FPED converts WWEIA foods to their respective number of cup equivalents of various food groups including dairy (including cheese). The cup equivalents of cheese per pizza food were converted to grams of cheese per 100 gram food for pizza codes that did not have a full recipe breakdown in the USDA recipes. Additionally, it was conservatively assumed that the cheese in the pizza was entirely mozzarella.

  - Turnovers: The amount of mozzarella cheese per 100 grams of food in cheese-filled turnovers was based on the average mozzarella amount per 100 grams of turnovers with complete USDA recipe breakdowns. It was conservatively assumed that the cheese in the cheese-filled turnovers was entirely mozzarella.
  - Lasagna: The amount of mozzarella cheese per 100 grams of food in lasagna foods was based on similar lasagna foods and USDA recipes that indicated the amount of mozzarella was approximately 7 grams per 100 grams food.

- Fermented milk: The amount of fermented milk per 100 grams of food in several buttermilk biscuits which had incomplete USDA recipe breakdowns was assumed to be 35% of the biscuit. This percentage was based on a similar food that had a recipe breakdown.
- Yogurt: As was done in determining the mozzarella amount in pizza, the amount of yogurt per 100 grams of food in coated snacks (i.e., bars, pretzel), candy not containing chocolate, and margarine products was also based on FPED 2011-2012 and 2009-2010.

Results

Two-day average thiocyanate intake estimates from the proposed use in five food categories were calculated based on food consumption data collected in NHANES 2009-2012. Both the *per capita* and *per user* mean and 90th percentile results for the total U.S. population in mg/day are provided in Table 2 from all proposed foods combined based on proposed use levels and total maximum levels (i.e., naturally occurring + proposed use levels).

Table 2. Two-day average estimated daily intake (EDI) of thiocyanate (mg/day) based on proposed and total maximum levels in milk-based foods by the total U.S. population; NHANES 2009-2012

	Total U.S. Population					
			Per Capita (mg/day)		Per User (mg/day)	
Thiocyanate EDI based on[1]	**Un-wtd N[2]**	**% User**	**Mean**	**90th Percentile**	**Mean**	**90th Percentile**
Proposed use levels	7,576	49	0.59	2.00	1.20	3.33
Total maximum levels (i.e., naturally occurring +proposed use)	10,208	67	1.63	5.52	2.44	7.24

[1] Thiocyanate use levels provided in Table 1.
[2] Unweighted number of users; % user, *per capita* and *per user* estimates for NHANES derived using the statistical weights provided by the NCHS.
Note: Baby foods were excluded from the analysis.

References

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Appendix I: Foods Included In Analysis

Food Category	Food code	Food description
Mozzarella	14010000	Cheese, NFS*
	14100100	Cheese, natural, NFS*
	14107010	Cheese, Mozzarella, NFS
	14107020	Cheese, Mozzarella, whole milk
	14107030	Cheese, Mozzarella, part skim
	14107040	Cheese, Mozzarella, reduced sodium
	14107060	Cheese, Mozzarella, nonfat or fat free
	14620300	Topping from cheese pizza*
	14620310	Topping from vegetable pizza*
	14620320	Topping from meat pizza*
	14620330	Topping from meat and vegetable pizza*
	14660200	Cheese, nuggets or pieces, breaded, baked, or fried*
	27135110	Veal parmigiana*
	27146300	Chicken or turkey parmigiana*
	27460510	Antipasto with ham, fish, cheese, vegetables*
	27510700	Meatball and spaghetti sauce submarine sandwich*
	28113110	Salisbury steak, baked, with tomato sauce, vegetable (diet frozen meal)*
	28140730	Chicken patty, breaded, with tomato sauce and cheese, fettuccine alfredo, vegetable (frozen meal)*
	58106200	Pizza, cheese, prepared from frozen, thin crust*
	58106205	Pizza, cheese, prepared from frozen, thick crust*
	58106210	Pizza, cheese, from restaurant or fast food, NS as to type of crust*
	58106220	Pizza, cheese, from restaurant or fast food, thin crust*
	58106225	Pizza, cheese, from restaurant or fast food, regular crust*
	58106230	Pizza, cheese, from restaurant or fast food, thick crust*
	58106233	Pizza, cheese, stuffed crust*
	58106235	Pizza, cheese, from school lunch, thin crust*
	58106236	Pizza, cheese, from school lunch, thick crust*
	58106250	Pizza, extra cheese, thin crust*
	58106255	Pizza, extra cheese, regular crust*
	58106260	Pizza, extra cheese, thick crust*
	58106300	Pizza, cheese, with vegetables, prepared from frozen, thin crust*
	58106305	Pizza, cheese with vegetables, prepared from frozen, thick crust*
	58106310	Pizza, cheese, with vegetables, NS as to type of crust*
	58106320	Pizza, cheese, with vegetables, thin crust*
	58106325	Pizza, cheese, with vegetables, regular crust*
	58106330	Pizza, cheese, with vegetables, thick crust*
	58106345	Pizza with cheese and extra vegetables, thin crust*
	58106347	Pizza with cheese and extra vegetables, regular crust*
	58106350	Pizza with cheese and extra vegetables, thick crust*
	58106358	Pizza, cheese, with fruit, thin crust*

58106359	Pizza, cheese, with fruit, regular crust* -
58106360	Pizza, cheese, with fruit, thick crust* -
58106411	Pizza with chicken, thin crust* -
58106412	Pizza with chicken, regular crust* -
58106413	Pizza with chicken, thick crust* -
58106441	Pizza with chicken and vegetables, thin crust* -
58106442	Pizza with chicken and vegetables, regular crust* -
58106443	Pizza with chicken and vegetables, thick crust* -
58106462	Pizza with chicken and fruit, regular crust* -
58106500	Pizza with meat, prepared from frozen, thin crust* -
58106505	Pizza with meat, prepared from frozen, thick crust* -
58106540	Pizza with pepperoni, from restaurant or fast food, NS as to type of crust* -
58106550	Pizza with pepperoni, from restaurant or fast food, thin crust* -
58106555	Pizza with pepperoni, from restaurant or fast food, regular crust* -
58106560	Pizza with pepperoni, from restaurant or fast food, thick crust* -
58106565	Pizza with pepperoni, stuffed crust* -
58106570	Pizza with pepperoni, from school lunch, thin crust* -
58106580	Pizza with pepperoni, from school lunch, thick crust* -
58106610	Pizza with meat other than pepperoni, from restaurant or fast food, NS as - to type of crust* -
58106620	Pizza with meat other than pepperoni, from restaurant or fast food, thin - crust* -
58106625	Pizza with meat other than pepperoni, from restaurant or fast food, regular - crust* -
58106630	Pizza with meat other than pepperoni, from restaurant or fast food, thick - crust* -
58106633	Pizza, with meat other than pepperoni, stuffed crust* -
58106635	Pizza, with meat other than pepperoni, from school lunch, thin crust* -
58106636	Pizza, with meat other than pepperoni, from school lunch, thick crust* -
58106640	Pizza with extra meat, NS as to type of crust* -
58106650	Pizza with extra meat, thin crust* -
58106655	Pizza with extra meat, regular crust* -
58106660	Pizza with extra meat, thick crust* -
58106700	Pizza with meat and vegetables, prepared from frozen, thin crust* -
58106705	Pizza with meat and vegetables, prepared from frozen, thick crust* -
58106710	Pizza with meat and vegetables, NS as to type of crust* -
58106720	Pizza with meat and vegetables, thin crust* -
58106725	Pizza with meat and vegetables, regular crust* -
58106730	Pizza with meat and vegetables, thick crust* -
58106733	Pizza with extra meat and extra vegetables, prepared from frozen, thin - crust* -
58106734	Pizza with extra meat and extra vegetables, prepared from frozen, thick - crust* -
58106735	Pizza with extra meat and extra vegetables, NS as to type of crust* -
58106736	Pizza with extra meat and extra vegetables, thin crust* -
58106737	Pizza with extra meat and extra vegetables, thick crust* -
58106738	Pizza with extra meat and extra vegetables, regular crust* -

	58106750	Pizza with meat and fruit, thin crust*
	58106755	Pizza with meat and fruit, regular crust*
	58106760	Pizza with meat and fruit, thick crust*
	58106820	Pizza with beans and vegetables, thin crust*
	58106910	Pizza with seafood, thin crust*
	58106915	Pizza with seafood, regular crust*
	58107220	White pizza, thin crust*
	58107225	White pizza, regular crust*
	58107230	White pizza, thick crust*
	58108000	Calzone, with cheese, meatless*
	58108010	Calzone, with meat and cheese*
	58108050	Pizza rolls*
	58126130	Turnover, meat- and cheese-filled, no gravy*
	58126150	Turnover, meat- and cheese-filled, tomato-based sauce*
	58126160	Turnover, cheese-filled, tomato-based sauce*
	58126290	Turnover, meat- and cheese-filled, lower in fat*
	58126300	Turnover, meat- and cheese-filled, tomato-based sauce, lower in fat*
	58126400	Turnover, filled with egg, meat and cheese*
	58130011	Lasagna with meat*
	58130013	Lasagna with meat, canned*
	58130020	Lasagna with meat and spinach*
	58130140	Lasagna with chicken or turkey*
	58130150	Lasagna, with chicken or turkey, and spinach*
	58130310	Lasagna, meatless*
	58130320	Lasagna, meatless, with vegetables*
	58133110	Manicotti, cheese-filled, no sauce*
	58133120	Manicotti, cheese-filled, with tomato sauce, meatless*
	58133130	Manicotti, cheese-filled, with meat sauce*
	58134110	Stuffed shells, cheese-filled, no sauce*
	58134120	Stuffed shells, cheese-filled, with tomato sauce, meatless*
	58134130	Stuffed shells, cheese-filled, with meat sauce*
	58134160	Stuffed shells, cheese- and spinach- filled, no sauce*
	58301020	Lasagna with cheese and sauce (diet frozen meal)*
	58301030	Veal lasagna (diet frozen meal)*
	58301110	Vegetable lasagna (frozen meal)*
	58301150	Zucchini lasagna (diet frozen meal)*
	58302050	Beef and noodles with meat sauce and cheese (diet frozen meal)*
	58304200	Ravioli, cheese-filled, with tomato sauce (diet frozen meal)*
	75412060	Eggplant parmesan casserole, regular*
	75412070	Eggplant with cheese and tomato sauce*
Cottage cheese	14200100	Cheese, cottage, NFS
	14201010	Cheese, cottage, creamed, large or small curd
	14201200	Cottage cheese, farmer's
	14202010	Cheese, cottage, with fruit
	14202020	Cheese, cottage, with vegetables
	14203010	Cheese, cottage, dry curd
	14203020	Cheese, cottage, salted, dry curd

	14204010	Cheese, cottage, lowfat (1-2% fat)
	14204020	Cheese, cottage, lowfat, with fruit*
	14610200	Cheese, cottage cheese, with gelatin dessert*
	14610210	Cheese, cottage cheese, with gelatin dessert and fruit*
	27212050	Beef and macaroni with cheese sauce (mixture)*
	28110660	Meatballs, Swedish, in gravy, with noodles (diet frozen meal)*
	53400200	Blintz, cheese-filled*
	53400300	Blintz, fruit-filled*
	53511500	Danish pastry, with cheese, fat free, cholesterol free*
	58122320	Knish, cheese (pastry filled with cheese)*
	58301080	Lasagna with cheese and meat sauce, reduced fat and sodium (diet frozen meal)*
	72125310	Palak Paneer or Saag Paneer (Indian)*
Frozen dairy desserts	11459990	Yogurt, frozen, NS as to flavor, NS as to type of milk
	11460000	Yogurt, frozen, flavors other than chocolate, NS as to type of milk
	11460100	Yogurt, frozen, chocolate, NS as to type of milk
	11460160	Yogurt, frozen, chocolate, lowfat milk
	11460170	Yogurt, frozen, flavors other than chocolate, lowfat milk
	11460190	Yogurt, frozen, NS as to flavor, nonfat milk
	11460250	Yogurt, frozen, flavors other than chocolate, with sorbet or sorbet-coated
	11460300	Yogurt, frozen, flavors other than chocolate, nonfat milk
	11460400	Yogurt, frozen, chocolate, nonfat milk, with low-calorie sweetener
	11460410	Yogurt, frozen, flavors other than chocolate, nonfat milk, with low-calorie sweetener
	11460430	Yogurt, frozen, chocolate, whole milk
	11460440	Yogurt, frozen, flavors other than chocolate, whole milk
	11461260	Yogurt, frozen, cone, flavors other than chocolate
	11461270	Yogurt, frozen, cone, flavors other than chocolate, lowfat milk
	11461280	Yogurt, frozen, cone, chocolate, lowfat milk
	11541110	Milk shake, homemade or fountain-type, chocolate*
	11541120	Milk shake, homemade or fountain-type, flavors other than chocolate*
	11541400	Milk shake with malt*
	11541500	Milk shake, made with skim milk, chocolate*
	11541510	Milk shake, made with skim milk, flavors other than chocolate*
	11542100	Carry-out milk shake, chocolate*
	11542200	Carry-out milk shake, flavors other than chocolate*
	13110000	Ice cream, NFS
	13110100	Ice cream, regular, flavors other than chocolate
	13110110	Ice cream, regular, chocolate
	13110120	Ice cream, rich, flavors other than chocolate
	13110130	Ice cream, rich, chocolate
	13110140	Ice cream, rich, NS as to flavor
	13110200	Ice cream, soft serve, flavors other than chocolate
	13110210	Ice cream, soft serve, chocolate
	13110220	Ice cream, soft serve, NS as to flavor

13110310	Ice cream, no sugar added, NS as to flavor
13110320	Ice cream, no sugar added, flavors other than chocolate
13110330	Ice cream, no sugar added, chocolate
13120050	Ice cream bar or stick, not chocolate covered or cake covered
13120100	Ice cream bar or stick, chocolate covered
13120110	Ice cream bar or stick, chocolate or caramel covered, with nuts
13120120	Ice cream bar or stick, rich chocolate ice cream, thick chocolate covering
13120121	Ice cream bar or stick, rich ice cream, thick chocolate covering
13120130	Ice cream bar or stick, rich ice cream, chocolate covered, with nuts
13120140	Ice cream bar or stick, chocolate ice cream, chocolate covered
13120300	Ice cream bar, cake covered
13120310	Ice cream bar, stick or nugget, with crunch coating
13120400	Ice cream bar or stick with fruit
13120500	Ice cream sandwich
13120550	Ice cream cookie sandwich
13120700	Ice cream cone with nuts, flavors other than chocolate
13120710	Ice cream cone, chocolate covered, with nuts, flavors other than chocolate
13120720	Ice cream cone, chocolate covered or dipped, flavors other than chocolate
13120730	Ice cream cone, no topping, flavors other than chocolate
13120750	Ice cream cone with nuts, chocolate ice cream
13120760	Ice cream cone, chocolate covered or dipped, chocolate ice cream
13120770	Ice cream cone, no topping, chocolate ice cream
13120780	Ice cream cone, chocolate covered, with nuts, chocolate ice cream
13120790	Ice cream sundae cone
13120800	Ice cream soda, flavors other than chocolate*
13120810	Ice cream soda, chocolate*
13121000	Ice cream sundae, NS as to topping, with whipped cream
13121100	Ice cream sundae, fruit topping, with whipped cream
13121300	Ice cream sundae, chocolate or fudge topping, with whipped cream
13122100	Ice cream pie, no crust
13130300	Light ice cream, flavors other than chocolate (formerly ice milk)
13130310	Light ice cream, chocolate (formerly ice milk)
13130330	Light ice cream, no sugar added, flavors other than chocolate
13130340	Light ice cream, no sugar added, chocolate
13130600	Light ice cream, soft serve, flavors other than chocolate (formerly ice milk)
13130610	Light ice cream, soft serve, chocolate (formerly ice milk)
13130620	Light ice cream, soft serve cone, flavors other than chocolate (formerly ice milk)
13130630	Light ice cream, soft serve cone, chocolate (formerly ice milk)
13130700	Light ice cream, soft serve, blended with candy or cookies
13135000	Ice cream sandwich, made with light ice cream, flavors other than chocolate
13135010	Ice cream sandwich, made with light chocolate ice cream
13136000	Ice cream sandwich, made with light, no sugar added ice cream
13140100	Light ice cream, bar or stick, chocolate-coated (formerly ice milk)
13140110	Light ice cream, bar or stick, chocolate covered, with nuts (formerly ice milk)

	13140450	Light ice cream, cone, NFS (formerly ice milk)
	13140500	Light ice cream, cone, flavors other than chocolate (formerly ice milk)
	13140550	Light ice cream, cone, chocolate (formerly ice milk)
	13140580	Light ice cream, no sugar added, cone, chocolate
	13140660	Light ice cream, sundae, soft serve, chocolate or fudge topping (without whipped cream) (formerly ice milk)
	13140680	Light ice cream, sundae, soft serve, not fruit or chocolate topping (without whipped cream) (formerly ice milk)
	13140700	Light ice cream, creamsicle or dreamsicle (formerly ice milk)
	13140900	Light ice cream, fudgesicle (formerly ice milk)
	13142000	Milk dessert bar or stick, frozen, with coconut
	13150000	Sherbet, all flavors
	13160150	Fat free ice cream, no sugar added, chocolate
	13160160	Fat free ice cream, no sugar added, flavors other than chocolate
	13160400	Fat free ice cream, flavors other than chocolate
	13160410	Fat free ice cream, chocolate
	13161000	Milk dessert bar, frozen, made from lowfat milk
	13161500	Milk dessert sandwich bar, frozen, made from lowfat milk
	13161520	Milk dessert sandwich bar, frozen, with low-calorie sweetener, made from lowfat milk
	13161600	Milk dessert bar, frozen, made from lowfat milk and low calorie sweetener
	13161630	Light ice cream, bar or stick, with low-calorie sweetener, chocolate-coated (formerly ice milk)
	13170000	Baked Alaska*
	53112000	Cake, ice cream and cake roll, chocolate*
	53112100	Cake, ice cream and cake roll, not chocolate*
	53366000	Pie, yogurt, frozen
	91611050	Ice pop filled with ice cream, all flavor varieties
	92510730	Fruit punch, made with soda, fruit juice, and sherbet or ice cream*
Fermented milk	11112130	Milk, cow's, fluid, acidophilus, 2% fat
	11115000	Buttermilk, fluid, nonfat
	11115100	Buttermilk, fluid, 1% fat
	11115200	Buttermilk, fluid, 2% fat
	11115300	Buttermilk, fluid, whole
	52101000	Biscuit, baking powder or buttermilk type, NS as to made from mix, refrigerated dough, or home recipe*
	52101100	Biscuit, baking powder or buttermilk type, made from mix*
	52102040	Biscuit, baking powder or buttermilk type, made from refrigerated dough*
	52103000	Biscuit, baking powder or buttermilk type, commercially baked*
	52104010	Biscuit, baking powder or buttermilk type, made from home recipe*
	53341500	Pie, buttermilk*
Flavored Milk Drinks	11511000	Milk, chocolate, NFS
	11511100	Milk, chocolate, whole milk-based
	11511200	Milk, chocolate, reduced fat milk-based, 2% (formerly "lowfat")

	11511300	Milk, chocolate, skim milk-based
	11511400	Milk, chocolate, lowfat milk-based
	11519040	Milk, flavors other than chocolate, NFS
	11519050	Milk, flavors other than chocolate, whole milk-based
	11519105	Milk, flavors other than chocolate, reduced fat milk-based
	11519200	Milk, flavors other than chocolate, lowfat milk-based
	11519205	Milk, flavors other than chocolate, skim-milk based
	11531000	Eggnog, made with whole milk
	11531500	Eggnog, made with 2% reduced fat milk (formerly eggnog, made with "2% lowfat" milk)
	11551050	Milk fruit drink
	11552200	Orange Julius
	11553000	Fruit smoothie drink, made with fruit or fruit juice and dairy products
	11553100	Fruit smoothie drink, NFS
	11560000	Chocolate-flavored drink, whey- and milk-based
Yogurt	11410000	Yogurt, NS as to type of milk or flavor
	11411010	Yogurt, plain, NS as to type of milk
	11411100	Yogurt, plain, whole milk
	11411200	Yogurt, plain, lowfat milk
	11411300	Yogurt, plain, nonfat milk
	11420000	Yogurt, vanilla, lemon, or coffee flavor, NS as to type of milk
	11421000	Yogurt, vanilla, lemon, or coffee flavor, whole milk
	11422000	Yogurt, vanilla, lemon, maple, or coffee flavor, lowfat milk
	11422100	Yogurt, vanilla, lemon, maple, or coffee flavor, lowfat milk, sweetened with low calorie sweetener
	11423000	Yogurt, vanilla, lemon, maple, or coffee flavor, nonfat milk
	11424000	Yogurt, vanilla, lemon, maple, or coffee flavor, nonfat milk, sweetened with low calorie sweetener
	11425000	Yogurt, chocolate, NS as to type of milk
	11426000	Yogurt, chocolate, whole milk
	11427000	Yogurt, chocolate, nonfat milk
	11430000	Yogurt, fruit variety, NS as to type of milk
	11431000	Yogurt, fruit variety, whole milk
	11432000	Yogurt, fruit variety, lowfat milk
	11432500	Yogurt, fruit variety, lowfat milk, sweetened with low-calorie sweetener
	11433000	Yogurt, fruit variety, nonfat milk
	11433500	Yogurt, fruit variety, nonfat milk, sweetened with low-calorie sweetener
	11446000	Fruit and lowfat yogurt parfait
	27116100	Beef curry*
	27120160	Pork curry*
	27130100	Lamb or mutton curry*
	27146150	Chicken curry*
	27150100	Shrimp curry*
	27150320	Fish curry*
	27213010	Biryani with meat*
	27243100	Biryani with chicken*

27516010	Gyro sandwich (pita bread, beef, lamb, onion, condiments), with tomato and spread*
32101530	Egg curry*
53104580	Cheesecake -type dessert, made with yogurt, with fruit*
53441210	Basbousa (semolina dessert dish)*
53540500	Breakfast bar, date, with yogurt coating*
53540902	Nature Valley Chewy Granola Bar with Yogurt Coating*
53710902	Nature Valley Chewy Granola Bar with Yogurt Coating*
53714230	Granola bar, oats, nuts, coated with non-chocolate coating*
54408250	Pretzel, yogurt-covered*
54430010	Yogurt chips*
58124500	Pastry, filled with potatoes and peas, fried*
63401015	Apple and grape salad with yogurt and walnuts*
75440600	Vegetable curry*
81103041	Margarine-like spread, made with yogurt, stick, salted*
81104011	Margarine-like spread, reduced calorie, about 40% fat, made with yogurt, tub, salted*
83115000	Yogurt dressing*
91708150	Yogurt covered fruit snacks candy, with added vitamin C*
91731150	Peanuts, yogurt covered*
91739600	Raisins, yogurt covered*

* Only the food category component for proposed thiocyanate food use was included in the analysis.
Note: Excludes baby foods.

SUBMISSION END