ORIGINAL SUBMISSION

Morgan Lewis

#688

Gary L. Yingling
Senior Counsel
+1.202.739.5610
gary.yingling@morganlewis.com

December 29, 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
5001 Campus Drive
College Park, MD 20740

Re: GRAS Notification for Sodium Thiocyanate for Use in the Lactoperoxidase System

Dear Dr. Mattia:

On behalf of Taradon Laboratory ("Taradon"), we are submitting under cover of this letter one paper copy and one eCopy of Taradon's generally recognized as safe ("GRAS") notification for its sodium thiocyanate. 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 sodium thiocyanate 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, as part of the lactoperoxidase system ("LPO system").

In many parts of the world, sodium thiocyanate has been used to protect dairy products as a part of the LPO system, particularly in remote areas where farmers are not in close proximity to the market. In the US, sodium thiocyanate is intended to be used as a processing aid as part of the LPO system 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. Sodium thiocyanate 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 LPO system, including sodium thiocyanate, 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.

Morgan, Lewis & Bockius LLP

1111 Pennsylvania Avenue, NW
Washington, DC 20004
United States

T +1.202.739.3000
F +1.202.739.3001

Pursuant to the regulatory and scientific procedures established by 21 C.F.R. § 170.36, this use of sodium thiocyanate 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 SODIUM THIOCYANATE FOR USE IN THE LACTOPEROXIDASE SYSTEM

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

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PART 1: SIGNED STATEMENTS AND CERTIFICATIONS

1. This GRAS notice is submitted in accordance with 21 C.F.R. Part 170, Subpart E.

2. Name and Address of Submitting Company:
   Taradon Laboratory
   Avenue Leon Champagne, 2
   B-1480 Tubize
   Belgium
   Tel: +32.495.51.90.64
   Fax: +32.2.390.93.86

3. Name of Notified Substance:
   Sodium thiocyanate

4. Intended Conditions of Use:
   a. List of foods and/or drinking water to be added to:
      Sodium thiocyanate is intended for use in the lactoperoxidase system. The lactoperoxidase system is used in fresh cheese including mozzarella and cottage cheeses, frozen dairy desserts, fermented milk, flavored milk drinks, and yogurt.

   b. Proposed levels of use:
      The lactoperoxidase system, for which the notified substance sodium thiocyanate is a component, 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 the lactoperoxidase system is as follows:

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

   c. Purpose of substance in the food product:
      Sodium thiocyanate is intended for use as a component of the lactoperoxidase system to extend the shelf life of fresh cheese including mozzarella and cottage cheeses, frozen dairy desserts, fermented milk, flavored milk drinks, and yogurt.

      In many parts of the world, the lactoperoxidase system 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 lactoperoxidase system 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, and 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 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 lactoperoxidase relies on the production of short-lived intermediary oxidation products of the thiocyanate ion, principally hypothiocyanite (OSCN-), derived from the substance that is the subject of this notification, sodium thiocyanate.

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 lactoperoxidase reaction. At the conclusion of treatment with the lactoperoxidase system, 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 lactoperoxidase system 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.

d. Subpopulation expected to consume product: (if appropriate):
No specific subpopulations are anticipated.

5. Statutory Basis for GRAS Conclusion:
The statutory basis for the GRAS conclusion for sodium thiocyanate is scientific procedures. Taradon Laboratory has assembled the scientific data to conclude that sodium thiocyanate is generally recognized as safe for use as a component of the lactoperoxidase system.

6. It is the view of Taradon Laboratory that the substance is not subject to premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on Taradon's conclusion that sodium thiocyanate is GRAS for the intended use as a component of the lactoperoxidase system to extend the shelf life of fresh cheese including mozzarella and cottage cheeses, frozen dairy desserts, fermented milk, flavored milk drinks, and yogurt.

7. Availability of Information for FDA Review: The data and information that are the basis for Taradon Laboratory's GRAS determination are available for FDA's review, and copies will be sent to FDA upon request, in either electronic format or by paper copy. Requests for copies and arrangements for review of materials cited herein may be directed to:

Gary L. Yingling
Morgan, Lewis and Bockius, LLP
1111 Pennsylvania Ave, NW
Washington, DC 20004

(202) 739-5610
gary.yingling@morganlewis.com

8. Exemptions from FOIA Disclosure:
The information provided in this application does not contain confidential or proprietary information, and therefore no FOIA exemptions are claimed.

9. Authorization to Share Trade Secrets with FSIS:
Should FDA find the need to share the information in this application with FSIS, Taradon has no objections.

10. Certification
On behalf of Taradon Laboratory, I certify that, to the best of my knowledge, the GRAS notice is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to me and Taradon Laboratory, and pertinent to the evaluation of the safety and GRAS status of the use of sodium thiocyanate for use as a component of the lactoperoxidase system to extend the shelf life of fresh cheese including mozzarella and cottage cheeses, frozen dairy desserts, fermented milk, flavored milk drinks, and yogurt.

Signed:
(b) (6)

12/29/16

Gary L. Yingling
Senior Counsel
Morgan, Lewis, and Bockius LLP

Date

PART 2: IDENTITY OF THE NOTIFIED SUBSTANCE

2.1. Chemical Name

The chemical name of the notified substance is sodium thiocyanate. The thiocyanate is intended for use as part of the lactoperoxidase system, which consists of a lactoperoxidase enzyme, a glucose oxidase enzyme, sodium thiocyanate, sucrose, and glucose.

2.2. Formula

The chemical formula of sodium thiocyanate is NaSCN.

The sodium thiocyanate will be a component of the lactoperoxidase system. The lactoperoxidase system (LPO system) use levels will be 300 ppm of a powder which will be used in 1 liter of the dairy products. The composition of the 300 ppm is:

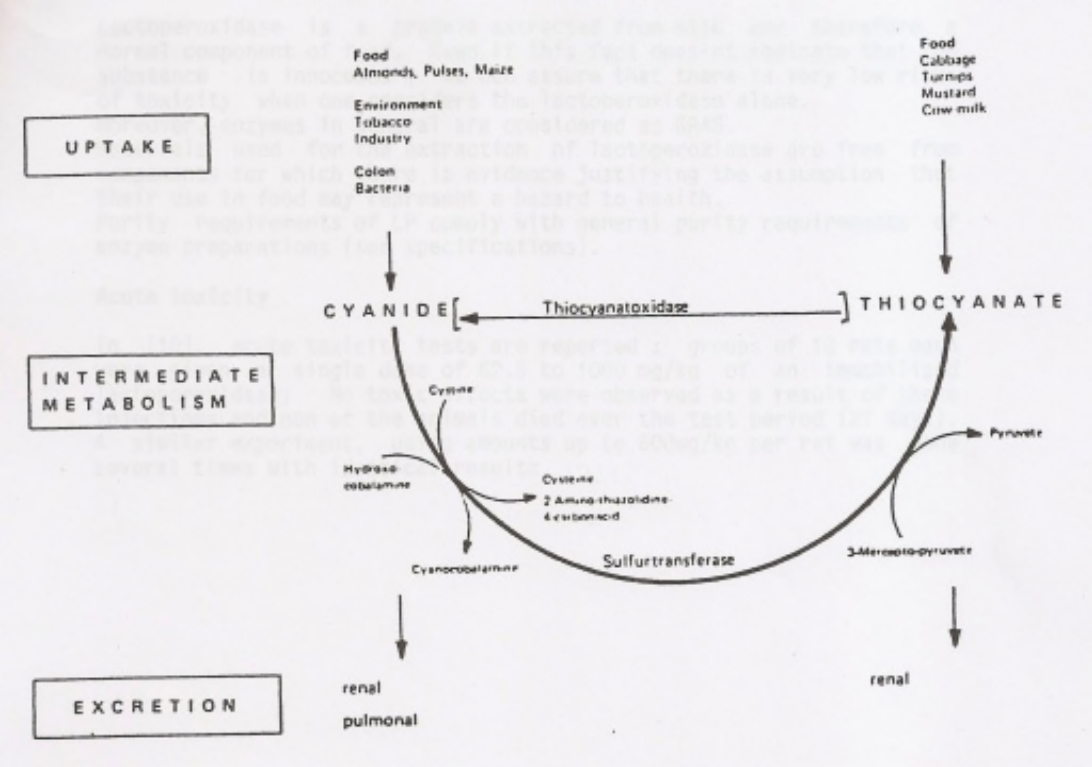
Lactoperoxidase: 1.25%
Glucose oxidase: 0.75%
Glucose: 30%
Sodium Thiocyanate: 5%
Sucrose: 63%

2.3. Composition

A. 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 LPO system use in dairy products, the proposed maximum levels of thiocyanate, the estimated intake of SCN^- for the consumers of lactoperoxidase system-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 LPO system-treated dairy products would appear greater than the background from general milk consumption. However, this does not take into account that in the LPO system, 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 LPO system is activated in a mixture, does not surpass the natural maximal concentration in any particular cow milk.

B. Lactoperoxidase System

Thiocyanate is intended for use as part of the lactoperoxidase (LPO) system. 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 sodium thiocyanate, for use in the lactoperoxidase system. The LPO system 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 LPO system – namely sucrose, glucose, and glucose oxidase, are GRAS ingredients for use in the foods. As glucose oxidase and glucose are well-defined GRAS substances, a detailed discussion of these components will be reserved. Reference is made to 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 that the Codex Alimentarius, where sodium thiocyanate and the lactoperoxidase system are the subject of a use guideline (Annex 1). The Food Chemical Codex also recognizes glucose oxidase as a food processing substance.

2.4. Specifications for food grade material

The sodium thiocyanate used in the production of the lactoperoxidase system is food grade, as are the remaining components of the LPO system. The certificates of analysis for three lots of sodium thiocyanate are included in Annex 2. All components will meet specifications and standards as required by the Food Chemical Codex, Codex Alimentarius, and regulations as set forth in 21 C.F.R. As sodium thiocyanate is intended for the purposes of this application for the exclusive use as a component of the lactoperoxidase system, the specification for the Taradon Laboratory lactoperoxidase system follows. The specification results of three batches are available in Annex 3, showing compliance of the production process with the specifications provided.

Physical/Chemical Specifications for the Taradon Laboratory Lactoperoxidase System:

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

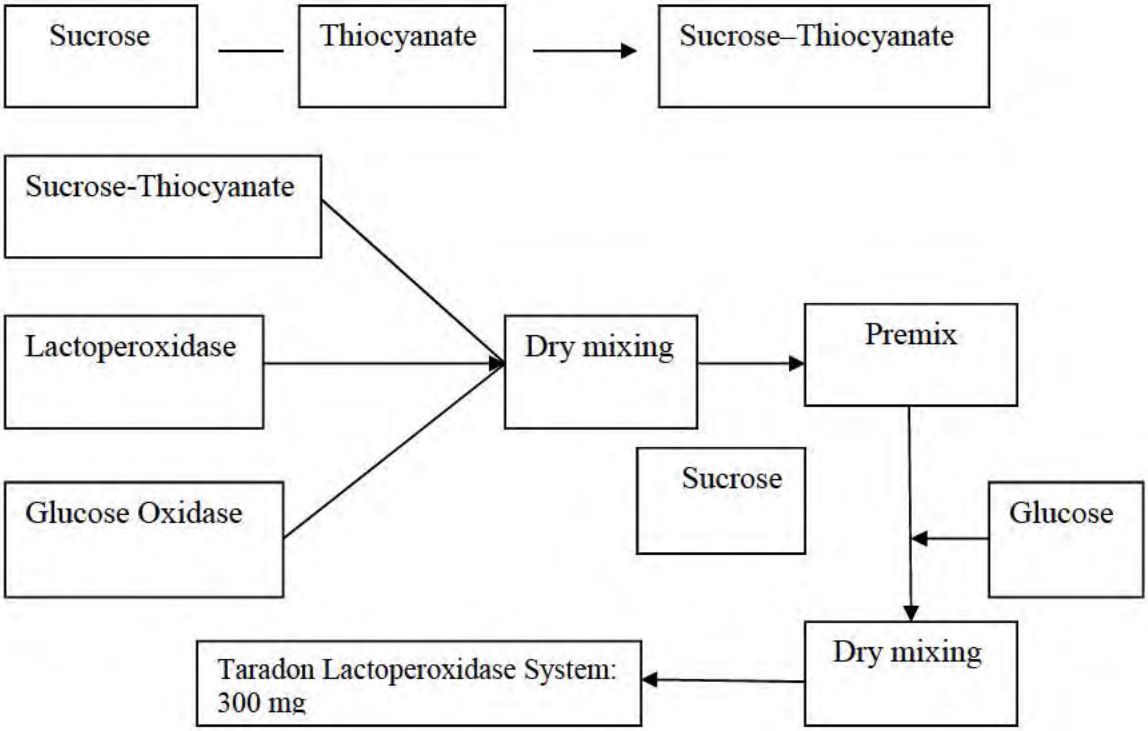
Sensory Specifications:

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

2.5. Method of Manufacture

As noted above, Taradon Laboratory does not manufacture the sodium thiocyanate, but instead uses the food grade sodium thiocyanate to manufacture the lactoperoxidase system. A schematic of the production of the Taradon lactoperoxidase system is shown below. Taradon's production and commercialization of enzymatic preparations has been certified from the British Retail Consortium.

Figure 1: Production Scheme



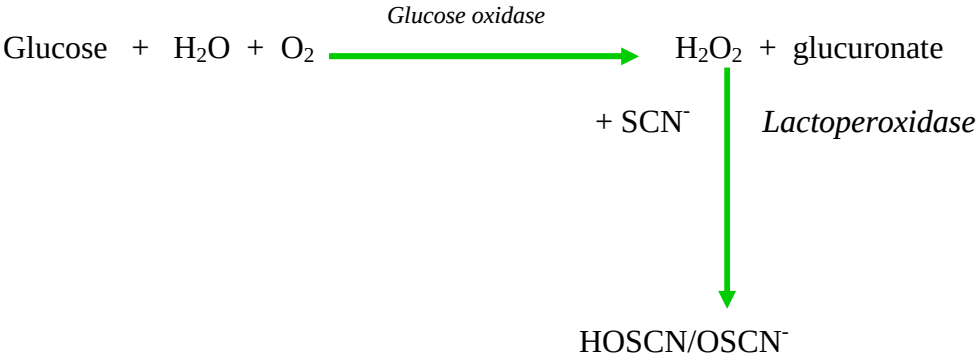
The ingredients of the lactoperoxidase system can be added to the desired product individually during production, or can be mixed to create the lactoperoxidase system prior to the start of the food production and stabilized with an inert support (such as sucrose), and added in the desired volume, during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

The product must be kept in a cool, dry place. The shelf-life of the lactoperoxidase system mixture 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

Sodium thiocyanate is a critical component of the lactoperoxidase system. The mode of action of the lactoperoxidase system relies on the production of short-lived intermediary oxidation products of the thiocyanate ion, principally the hypothiocyanite ions (OSCN-). The lactoperoxidase system 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 overall reaction is as follows:



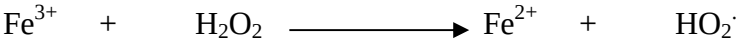
These $OSCN^-$ ions in turn reacts with the bacterial cytoplasmic membranes, as well as impair the function of metabolic enzymes, hence their antimicrobial effects (Mickelson, 1977; Reiter and Marshall 1979)

These $OSCN^-$ ions have a short-lived intermediary (+/- 400 minutes) after the starting of the LPO system reaction. Due to the short-lived of these ions, the LPO system 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 (LP), 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:



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).

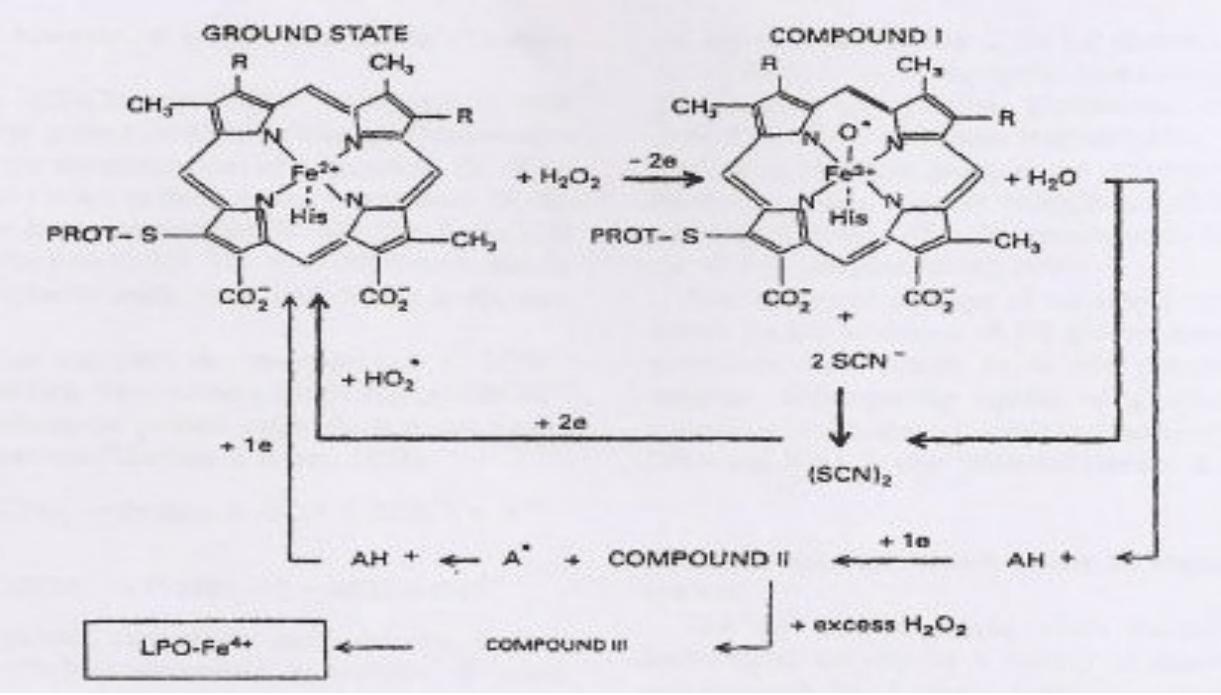


Figure 5: Pathways in the lactoperoxidase-catalyzed reaction mechanism. The normal peroxidalic cycle includes compound I. Insufficient 2-electron donors lead to compound II, and excess of H_2O_2 results in the formation of compound III (de Wit and van Hooydonck, 1996)

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:

Compound I + X ⟶ 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 LPO system 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 lactoperoxidase 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 lactoperoxidase 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 lactoperoxidase 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 lactoperoxidase 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 lactoperoxidase 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 lactoperoxidase system would result in any microbiological risks, e.g. development of lactoperoxidase system 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 LPO system is not likely to stimulate the development of resistance to the lactoperoxidase system itself or antibiotic-resistant microorganisms (Annex 4). This report is discussed in further detail in section 3.2.

2.8. Potential Toxicants

There are no potential toxicants from the sodium thiocyanate added as a processing aid in itself, as it produces unstable intermediates that decompose rapidly before consumption. It is important to consider the LPO system, however, as a reaction product of the 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 lactoperoxidase system 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 LPO system (Annex 5). 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.

PART 3: DIETARY EXPOSURE

3.1 Estimated Dietary Intake

As noted previously, thiocyanate will be used as a component of the LPO system, along with glucose, sucrose, and glucose oxidase. 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 6. 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.

PART 4: SELF-LIMITING LEVELS OF USE

The thiocyanate level of 5% of the 300 mg of LPO system is the maximum recommended dose. At concentrations exceeding this level, the taste and texture of the product may be impacted. The concern at higher levels is the taste and texture alterations; there is no issue of safety.

PART 5: EXPERIENCE BASED ON COMMON EXPERIENCE IN FOOD BEFORE 1958

This section is not applicable to this application.

PART 6: NARRATIVE

6.1. History of Safe Use

Thiocyanate, as part of the lactoperoxidase system, 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 lactoperoxidase system 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 LPO system 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 lactoperoxidase system 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 lactoperoxidase system 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 lactoperoxidase system 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 LPO system, 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 lactoperoxidase system 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 LPO system 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 LPO system 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 sodium thiocyanate as part of the lactoperoxidase system in dairy products.

6.2. Summary of Literature

6.2.1. FAO/WHO Technical Report

In 2005, the FAO/WHO Technical Meeting was held to evaluate the use of thiocyanate as a component of the lactoperoxidase system for preservation of raw milk. The resultant report of that meeting discusses in detail the lactoperoxidase system, as well as the potential risks and benefits of its use. This report is included in Annex 4.

The report discusses the efficacy of the lactoperoxidase system, 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 lactoperoxidase system does not promote microbial growth or encourage resistance. Further, the report also clearly states that use of the lactoperoxidase system 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 LPO system, and the report includes an extensive review of the literature pertaining to the use of the LPO system 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 LPO system 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 LPO system.

The report concluded that the LPO system 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.

6.2.2. 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 LPO system and thiocyanate in dairy products. The referenced scientific articles are provided in Annex 7.

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 LPO system 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 LPO system against milk spoilage organisms (Bjorck, et al., 1975). The authors determined that the LPO system 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 LPO system components have been removed.

Two studies have also evaluated the efficacy of the LPO system against *Listeria monocytogenes*. The first used a model broth culture system, and found that against the strain Scott A, LPO system 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 LPO system has a bactericidal effect, but this effect is strain and temperature dependent (Gaya, Medina, and Nunez, 1991). The LPO system 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 LPO system 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 LPO system, as well as an overview of the efficacy studies performed with the LPO system (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 LPO system was also bactericidal and bacteristatic against Staph aureus, a major cause of bovine infections as well as human infections. The LPO system 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 LPO system 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 LPO system, specifically focusing on the antimicrobial activity (Bafort, et al., 2014). Both of these review articles support the efficacy of the LPO system 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 LPO system to extend the shelf life of raw milk. The LPO system 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 LPO system for the intended use.

6.3. Toxicology Studies

The toxicity of thiocyanate was evaluated as a single ingredient, as well as in the LPO system. This evaluation is important, as the sodium thiocyanate is intended for use as a part of the system, and is consumed as a part of the reaction. As explained previously, the antibacterial effect of the LPO system 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 LPO system 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 LPO system 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 LPO system 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 LPO system 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 LPO system 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 LPO system (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 LPO system, 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 LPO system 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 LPO system 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 LPO system: 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 LPO system 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 LPO system 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 LPO system 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.

6.4. GRAS Conclusion

The information submitted as part of this GRAS notice demonstrates that sodium thiocyanate is GRAS for the proposed intended use as a component of the lactoperoxidase system. Three of the ingredients of the system are present in the human and animal body, including thiocyanate, the subject of this notice, which is present in human saliva and gastric juice. The chemical reaction of the LPO system 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 sodium thiocyanate is generally recognized as safe as a processing aid as part of the lactoperoxidase system for use in fresh cheese including mozzarella and cottage cheeses, frozen dairy desserts, fermented milk, flavored milk drinks, and yogurt.

PART 7: LIST OF REFERENCES

Pursuant to 21 C.F.R. 170.255, the list of supporting data and information referenced in the GRAS notice is contained below.

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LIST OF ANNEXES

Annex 1

GUIDELINES FOR THE PRESERVATION OF RAW MILK BY USE OF THE LACTOPEROXIDASE SYSTEM

CAC/GL 13-1991

INTRODUCTION

Milk is an easily perishable raw material. Contaminating bacteria may multiply rapidly and render it unsuitable for processing and/or unfit for human consumption. Bacterial growth can be retarded by refrigeration, thereby slowing down the rate of deterioration. Under certain conditions refrigeration may not be feasible due to economical and/or technical reasons. Difficulties in applying refrigeration are specially a problem for certain areas in countries setting up or expanding their milk production. In these situations, it would be beneficial to have access to a method, other than refrigeration, for retarding bacterial growth in raw milk during collection and transportation to the dairy processing plant.

In 1967 the FAO/WHO Expert Panel on Milk Quality concluded that the use of hydrogen peroxide might be an acceptable alternative in the early stages of development of an organized dairy industry, provided that certain conditions were complied with. However, this method has not achieved any general acceptance as it has several drawbacks, most important of which is the difficulty of controlling its use: it may be misused to disguise milk of basic hygienic quality produced under poor hygienic conditions. The toxicological aspects of the use of relatively high concentrations of hydrogen peroxide in milk have also been questioned.

A chemical method for preserving milk would still be of great advantage in certain situations. The search for such a method has therefore continued. Interest has recently been focused on the indigenous antibacterial systems in milk to determine if these could be applied practically to preserve raw milk. During the last decade, basic and applied research has demonstrated that one of these systems, the lactoperoxidase/thiocyanate/hydrogen peroxide system (LP-system) can be used successfully for this purpose.

1. SCOPE

1.1 This Code of Practice describes the use of the lactoperoxidase system for preventing bacterial spoilage of raw milk (bovine and buffalo) during collection and transportation to a dairy processing plant. It describes the principles of the method, in what situations it can be used, its practical application and control of the method. It should be stressed that this method should be utilized when refrigeration of the raw milk is not feasible.

2. PRINCIPLES OF THE METHOD

2.1 The lactoperoxidase/thiocyanate/hydrogen peroxide system is an indigenous antibacterial system in milk and human saliva. The enzyme lactoperoxidase is present in bovine and buffalo milk in relatively high concentrations. It can oxidise thiocyanate

ions in the presence of hydrogen peroxide. By this reaction, thiocyanate is converted into hypothiocyanous acid (HOSCN). At the pH of milk HOSCN is dissociated and exists mainly in the form of hypothiocyanate ions ($OSCN^-$). This agent reacts specifically with free sulphydryl groups, thereby inactivating several vital metabolic bacterial enzymes, consequently blocking their metabolism and ability to multiply. As milk proteins contain very few sulphydryl groups and those that are present are relatively inaccessible to $OSCN^-$ (masked), the reaction of this compound is in milk quite specific and is directed against the bacteria present in the milk.

2.2 The effect against bacteria is both species and strain dependent. Against a mixed raw milk flora, dominated by mesophilic bacteria, the effect is bacteriostatic (predominantly inhibitory). Against some gram-negative bacteria, i.e. pseudomonads, *Escherichia coli,* the effect is bactericidal. Due to the mainly bacteriostatic effect of the system it is not possible to disguise poor quality milk, which originally contained a high bacterial population, by applying this method.

2.3 The antibacterial oxidation products of thiocyanate are not stable at neutral pH. Any surplus of these decomposes spontaneously to thiocyanate. The velocity of this reaction is temperature dependent, i.e. more rapid at higher temperatures. Pasteurisation of the milk will ensure a complete removal of any residual concentrations of the active oxidation products.

2.4 Oxidation of thiocyanate does not occur to any great extent in milk when it has left the udder. It can, however, be initiated through addition of small concentrations of hydrogen peroxide (see Section 4). The high concentrations of hydrogen peroxide used to preserve milk (300–800 ppm), destroy the enzyme lactoperoxidase and thereby preclude the oxidation of thiocyanate. With this method the antibacterial effect is thus an effect of hydrogen peroxide itself.

2.5 The antibacterial effect of the LP-system is, within certain limits, proportional to the thiocyanate concentration in the milk (provided that an equimolar amount of hydrogen peroxide is provided). The level thiocyanate in milk is related to the feeding of the animals and can thus vary. The practical use of the method consequently requires addition of some thiocyanate to ensure that a level necessary to achieve the desired effect, is present in the milk.

2.6 The levels of thiocyanate resulting from this treatment are within the physiological levels reported to occur in milk under certain circumstances and feeding regimes. They are also far below the thiocyanate levels known to exist in human saliva and certain common vegetables, e.g. cabbage and cauliflower. In addition, results from clinical experiments have clearly demonstrated that milk treated according to this method will not cause any interference of the iodine uptake of the thyroid gland, neither in persons with a normal iodine status nor in cases of iodine deficiency.

3. INTENDED UTILIZATION OF METHOD

3.1 This method should only be used in situations when technical, economical and/or practical reasons do not allow the use of cooling facilities for maintaining the quality of raw milk. Use of the LP-system in areas which currently lack an adequate infrastructure for collection of liquid milk, would ensure the production of milk as a safe and wholesome food, which otherwise would be virtually impossible.

3.2 The method should not be used by the individual farmers but at a suitable collecting point/centre. These centres must be equipped with proper facilities for cleaning and sanitising the vessels used to hold and transport milk.

3.3 The personnel responsible for the collection of milk should be in charge for the treatment of the milk. They should be given appropriate training, including training in general milk hygiene, to enable them fulfil this in a correct way.

3.4 The dairy processing the milk collected by use of the lactoperoxidase system should be made responsible for ensuring that the method is used as intended. This dairy should set up appropriate control methods (see Section 5) to monitor usage of the method, raw milk quality and quality of the milk prior to processing.

3.5 The method should primarily be used to prevent undue bacterial multiplication in raw milk during collection and transportation to the dairy processing plant under conditions stated in 3.1. The inhibitory effect of the treatment is dependent on the temperature of the stored milk and has been found to act for the following periods of time in laboratory and field-experiments carried out in different countries with raw milk of an initial good hygienic standard:

Temperature, C	Time, h
30	7–8
25	11–12
20	16–17
15	24–26

3.6 The use of the lactoperoxidase method does not exclude the necessity of pasteurization of the milk before human consumption. Neither does it exclude the normal precautions and handling routines applied to ensure a high hygienic standard of the raw milk.

4. PRACTICAL APPLICATION OF THE METHOD

4.1 The lactoperoxidase system can be activated in raw milk to give the above stated antibacterial effect by an addition of thiocyanate as sodium thiocyanate and hydrogen peroxide in the form of sodium percarbonate by the following procedure:

- 14 mg of NaSCN is added per litre of milk. The milk should then be mixed to ensure an even distribution of the SCN-. Plunging for about 1 minute with a clean plunger is normally satisfactory.
- Secondly, 30 mg of sodium percarbonate is added per litre of milk. The milk is then stirred for another 2–3 minutes to ensure that the sodium percarbonate is completely dissolved and the hydrogen peroxide is evenly distributed in the milk.

4.2 It is essential that the sodium thiocyanate and sodium percarbonate are added in the order stated above. The enzymatic reaction is started in the milk when the hydrogen peroxide (sodium percarbonate) is added. It is completed within about 5 minutes from the addition of H_2O_2; thereafter, no hydrogen peroxide is present in the milk.

4.3 The activation of the lactoperoxidase system should be carried out within 2–3 hours from the time of milking.

4.4 Quantities of sodium thiocyanate and sodium percarbonate needed for the treatment of a certain volume of milk, for example 40 or 50 litre milk churns, should be distributed to the collecting centre/point in prepacked amounts lasting for a few weeks at a time. The technical specifications of the thiocyanate and sodium percarbonate which should be used are stated in Appendices I and II.

5. CONTROL OF USAGE

5.1 The use of the lactoperoxidase system for preserving raw milk must be controlled by the dairy processing plant receiving the milk. This should be a combination of currently used acceptance tests, e.g. titratable acidity, methylene blue, resazurin, total viable count and analyses of the thiocyanate concentration in the milk. Since the thiocyanate is not consumed in the reaction, treated milk arriving at the dairy plant would contain approximately 10 mg above the natural amount of thiocyanate (the latter can be determined by analysing untreated milk from the same area) per litre of milk. The analytical method for SCN^- is described in Appendix III Testing should be undertaken at random. If the concentration of thiocyanate is too high (or too low), investigation must be carried out to determine why the concentration is outside specification. The dairy processing plant should also be responsible for the control of the chemicals to be used at the collection centre for the activation of the lactoperoxidase system.

5.2 Analysis of the bacteriological quality of the milk (methylene blue, resazurin, total plate count) should also be carried out to ensure that good hygienic standards are not neglected. Since the effects of the system are predominantly bacteriostatic, an initial high bacterial population in the milk can still be revealed by such tests.

APPENDIX I

TECHNICAL SPECIFICATION OF SODIUM THIOCYANATE

Definition

Chemical name	Sodium thiocyanate
Chemical formula	NaSCN
Molecular weight	81.1
Assay content	98–99%
Humidity	1–2%

Purity (according to JECFA* specification)

Heavy metals (as Pb)	< 2 ppm
Sulfates (as SO_4)	< 50 ppm
Sulfide (S)	< 10 ppm

* Joint FAO/WHO Expert Committee on Food Additives.

APPENDIX II

TECHNICAL SPECIFICATION OF SODIUM PERCARBONATE

Definition

Chemical name	Sodium percarbonate (*)
Chemical formula	$2Na_2CO_3 \cdot 3H_2O_2$
Molecular weight	314.0
Assay content	85%

Commercial available sodium percarbonate recommended to be used has the following specification:

Sodium carbonate peroxyhydrate	> 85%
Heavy metals (as Pb)	< 10 ppm
Arsenic (as As)	< 3 ppm

(*) For information where sodium percarbonate could be obtained commercially, please apply to IDF General Secretariat, Silver Building, Blvd. A. Reyers 70/B, B-1030 Brussels, Belgium.

APPENDIX III

ANALYSIS OF THIOCYANATE IN MILK

Principle

Thiocyanate can be determined in milk, after deproteinisation, with trichloroacetic acid (TCA), as the ferric complex by measuring the absorbance at 460 nm. The minimum level of detection by this method is 1 to 2 ppm of SCN^- .

Reagent solutions

1. 20% (w/v) trichloroacetic acid: 20 g TCA is dissolved in 100 ml of distilled water and filtered.
2. Ferric nitrate reagent: 16.0 g $Fe(NO_3)_3.9H_2O$ is dissolved in 50 ml 2 M HNO_3* and then diluted with distilled water to 100 ml. The solution should be stored dark and cold.

   * 2M HNO_3 is obtained by diluting 138.5 ml 65% HNO_3 to 1 000 ml with distilled water.

Determination

4.0 ml of milk is mixed with 2.0 ml of 20% TCA solution. The mixture is blended well and then allowed to stand for at least 30 minutes. It is thereafter filtered through a suitable filter paper (Whatman No. 40). 1.5 ml of the clear filtrate is then mixed with 1.5 ml of the ferric nitrate reagent and the absorbance measured at 460 nm. As a blank, a mixture of 1.5 ml of ferric nitrate solution and 1.5 ml of water is used. The measurement must be carried out within 10 minutes from the addition of the ferric nitrate solution as the coloured complex is not stable for any length of time. The concentration of thiocyanate is then determined by comparison with standard solutions of known thiocyanate concentration, e.g. 10, 15, 20 and 30 µg/ml of thiocyanate.

Annex 2

SODIUM THIOCYANATE

Certificate of Analysis

Delivery Address

Order item	5100455684 000020
Delivery item	6100587611 000020
Material number	5404306
Customer ref.	

Analysis

Batch number 1403070719

Quantity

Characteristic	Unit	Values	Spec Limits min.	Spec Limits max.	Method of Analysis
Appearance	-	White crystals			
Assay (on dried basis)	%	99,5	98,0	-	AG/89.1
pH (5%-Solution)	-	6,3	5,0	9,0	F/89.2
Iron	mg/kg	0,1	-	3,0	KOL 92/02

Our certificates of analysis are based on analyses carried out on random samples taken from the production batches from which you have been supplied. Thi certificate of analysis does not exempt you from testing the suitability of the delivered product for your applications.

Date:18.07.2014

Approved by:

Thomas Schauzu
(QHSSE Manager)

Carbosulf Chemische Werke GmbH
Geestemünder Str. 26
D-50735 Köln
Germany
Phone +49 221 7496-0
Fax +49 221 7496-190

This Certificate of Analysis has been produced electronically and is effective without a signature.

CARBOSULF

SODIUM THIOCYANATE

Certificate of Analysis

Delivery Address

Order item	5100562161 000020
Delivery item	6100724415 000020
Material number	5404306
Customer ref.	

Analysis

Batch number	1501071319
Quantity	

Characteristic	Unit	Values	Spec Limits min.	Spec Limits max.	Method of Analysis
Appearance	-	White crystals	-	-	
Assay (on dried basis)	%	99,9	98,0	-	AG/89.1
pH (5%-Solution)	-	6,1	5,0	9,0	F/89.2
Iron	mg/kg	0,1	-	3,0	KOL 92/02

Our certificates of analysis are based on analyses carried out on random samples taken from the production batches from which you have been supplied. This certificate of analysis does not exempt you from testing the suitability of the delivered product for your applications.

Date:05.02.2015

Approved by:

Thomas Schauzu
(QHSSE Manager)

Carbosulf Chemische Werke GmbH
Geestemünder Str. 26
D-50735 Köln
Germany
Phone +49 221 7496-0
Fax +49 221 7496-190

This Certificate of Analysis has been produced electronically and is effective without a signature.

CARBOSULF

SODIUM THIOCYANATE

Certificate of Analysis

Delivery Address

Order item 5100976166 000020
Delivery item 6101263638 000020
Material number 5404306

Customer ref.

Analysis
Batch number 1606070619
Quantity

Characteristic	Unit		Spec Limits min.	max.	Method of Analysis
Appearance	-	White crystals			
Assay (on dried basis)	%	99,9	99,0	-	AG/89.1
pH (5%-Solution)	-	5,7	5,0	9,0	F/89.2
Iron	mg\kg	0,1	-	3,0	KOL 92/02

Our certificates of analysis are based on analyses carried out on random samples taken from the production batches from which you have been supplied. This certificate of analysis does not exempt you from testing the suitability of the delivered product for your applications.

Date:06.07.2016

Approved by:

Thomas Schauzu
(QHSSE Manager)

Carbosulf Chemische Werke GmbH
Geestemünder Str. 26
D-50735 Köln
Germany
Phone +49 221 7496-0
Fax +49 221 7496-190

This Certificate of Analysis has been produced electronically and is effective without a signature.

Carbosulf
Chemische Werke GmbH
(Subsidiary of Akzo Nobel Functional Chemicals GmbH)
Geestemünder Str. 26
50735 Köln
Germany
T +49 (0)221-7496-0
F +49 (0)221-7496-190

AkzoNobel

Product Specification

Sodium Thiocyanate crystalline

Chemical Name:	Sodium thiocyanate
Molecular Formula:	NaSCN
Molecular Mass:	81,1 g/mol
CAS-No.:	540-72-7
EC-No.:	208-754-4

Properties

Bulk density:	approx. 750 kg/m^3
Solubility in water (20°C):	approx. 1250 g/l
Melting point:	approx. 310 °C

Specification

Appearance:	white crystals
Content (on dried basis):	min. 99,0 %
Moisture:	max. 3,0 %
Iron:	max. 3 mg/kg
pH (5% aqueous solution):	5,0 – 9,0
Ammonia (as NH_3)	max. 200 mg/kg

Typical Characteristics

Chloride:	< 200 mg/kg
Sulphate:	< 300 mg/kg
Heavy metals:	< 10 mg/kg

Analytical methods are available on request.

Major Applications

In the fiber industry in spinning baths for acrylic fibers.
In the water treatment industry as corrosion inhibitor.
In agriculture as an intermediate in the manufacture of herbicides.
In the photographic industry as sensitizer and stabilizer.
In concrete industry as hardening accelerator.

Storage

Store in a cool and dry place and avoid any contact to a strong acid.
Use resistant equipment like polymer materials and high grade alloys. Iron corrosion can result in red coloration of product when exposed to UV-light. Although the product is stable when stored under ambient conditions without exposure to other chemicals, it is advised to re-analyze before use after 3 years of storage. Thiocyanates are hygroscopic and will attract humidity from air. This might result in higher moisture content in the product after some time.

Packing and Transport

Sodium thiocyanate is delivered in:	25 kg net in paper bags
Hazard Identification No.:	none
UN-No.:	none

Safety advice

For transport, handling and first aid instructions we refer to our Material Safety Data Sheet (MSDS).

Issue June 2015/T. Morris

Annex 3

TARADON LPO-SYSTEM

CERTIFICATE OF ANALYSIS

Lot: CS-DON 20161024

Per gr of powder

Lactoperoxidase activity:	12,500 ABTS units
Glucose Oxydase activity:	325 ABTS units
Na-Thiocyanate :	50 mg
Glucose:	300 mg
$OSCN^-$ production:	> 100 µM

Bacterial count:

Total plate count	< 10 CFU / g
E. coli	Absent /g
Yeast and Molds	< 10 CFU / g
Staphylococcus aureus	Absent / 2gr
Salmonella	Absent / 5gr

Manufaturing date : 24/10/2016
Expired date : 24/10/2017

Jean-Paul Perraudin
Quality Control Coordinator

TARADON LABORATORY - Av. Léon Champagne, 2 - B-1480 Tubize (Saintes) - Belgium
Tel +32 (0)476 84 27 62 - E-mail : jb.morel@taradon-laboratory.com

TARADON LPO SYSTEM

CERTIFICATE OF ANALYSIS

Lot: CS-DON 20160818

Per gr of powder

Lactoperoxidase activity:	12,500 ABTS units
Glucose Oxydase activity:	310 ABTS units
Na-Thiocyanate :	50 mg
Glucose:	305 mg
$OSCN^-$ production:	> 100 µM

Bacterial count:

Total plate count	< 10 CFU / g
E. coli	Absent /g
Yeast and Molds	< 10 CFU / g
Staphylococcus aureus	Absent / 2gr
Salmonella	Absent / 5gr

Manufaturing date : 18/08/2016
Expired date : 18/08/2017

Jean-Paul Perraudin
Quality Control Coordinator

TARADON LPO-SYSTEM

CERTIFICATE OF ANALYSIS

Lot: CS-DON 20151028

Per gr of powder

Lactoperoxidase activity:	12,625 ABTS units
Glucose Oxydase activity:	315 ABTS units
Na-Thiocyanate :	50 mg
Glucose:	300 mg
$OSCN^-$ production:	> 100 µM

Bacterial count:

Total plate count	< 10 CFU / g
E. coli	Absent /g
Yeast and Molds	< 10 CFU / g
Staphylococcus aureus	Absent / 2gr
Salmonella	Absent / 5gr

Manufaturing date : 28/10/2015
Expired date : 28/10/2016

Jean-Paul Perraudin
Quality Control Coordinator

Annex 4

Benefits and Potential Risks of the Lactoperoxidase system of Raw Milk Preservation

Report of an FAO/WHO technical meeting

O Headquarters, Rome, Italy, 28 November - 2 December, 2005

World Health Orga ı i

FAO FIAT PANIS

For further information, please contact:

Food Quality and Standards Service
Nutrition and Consumer Protection Division
Food and Agriculture Organization of the United Nations
Viale delle Terme di Caracalla
I-00153 Rome, Italy

Fax: +39 06 57054593
E.mail: jemra@fao.org

or

Animal Production Service
Animal Production and Health Division
Food and Agriculture Organization of the United Nations
Viale delle Terme di Caracalla
I-00153 Rome, Italy

Fax: +39 06 57055749

Web sites:
http://www.fao.org/ag/agn/
http://www.fao.org/ag/dairy.html

Cover picture:

E.mail: drorbn@math.toronto.edu

Benefits and Potential Risks of the Lactoperoxidase System of Raw Milk Preservation

Report of an FAO/WHO technical meeting
FAO Headquarters, Rome, Italy
28 November - 2 December 2005

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Contents

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Appreciation is also extended to all those who responded to the call for data that was issued by FAO and WHO and brought to our attention information that was not readily available in the mainstream literature and official documentation.

The preparatory work and technical meeting convened to prepare this report was coordinated by the Animal Production and Food Quality and Standards Services of FAO. This included Anthony Bennett, Sarah Cahill, Ruth Charrondiere, Maria de Lourdes Costarrica, Frederic Lhoste and Simon Mack in FAO and Hae Jung Joon and Jørgen Schlundt in WHO. Publication of the report was co-ordinated by Anthony Bennett, Sarah Cahill and Frederic Lhoste. Art direction by Nicoletta Forlano and design by James Morgan. James Edge edited the report.

The work was supported and funded by the Animal Production and the Food Quality and Standards Services of FAO and the WHO Department of Food Safety, Zoonoses and Foodborne Disease.

Meeting participants

Prof. Olivia Calisay EMATA
Assistant Professor and University Researcher
Dairy Training and Research Institute
ADSC. UP Los Baños College
Laguna, Philippines

Dr Alistair GRANDISON
Senior Lecturer
The University of Reading
United Kingdom

Prof. Hannu Jaakko Tapani KORHONEN
Director of Food Research
MTT Agrifood Research
Finland

Prof. Christiaan Wilfried MICHIELS
Professor in Food Microbiology and Head of the Laboratory of Food Microbiology
Katholieke Universiteit Leuven
Belgium

Mr Hezekiah Gichere MURIUKI
Dairy Economics Consultant
Kenya

Ing. Pastor Ceballo PONCE
Director of the Center of Assays for the Control and Quality of Milk and Dairy Products (CENLAC) of the National Center for Animal and Plant Health (CENSA)
Cuba

Prof. Jean Paul RAMET
Professor of Food Science and Technology (Retired)
Ecole Nationale Supérieure d'Agronomie et des Industries Alimentaires
France

Dr John VANDERVEEN
Emeritus Scientist, Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
United States of America

Prof. Ronald WALKER
Emeritus Professor of Food Science
University of Surrey
United Kingdom

Declarations of interest

Ing. Ponce: As a researcher at the National Centre for Animal and Plant Health (CENSA), he is the author of a patent on a product based on the activation of the Lactoperoxidase system. He does not have rights for its commercial exploitation nor profits derived from it as established by Cuban Laws of Intellectual Property.

Abbreviations

CAC	Codex Alimentarius Commission
CCFH	Codex Committee on Food Hygiene
COMESA	Common Market for Eastern and Southern Africa
FAO	Food and Agriculture Organization of the United Nations
GEMS	Global Environment Monitoring System
GLP	The FAO Global Lactoperoxidase Experts Group
JECFA	Joint FAO/WHO Expert Committee on Food Additives
HTST	High Temperature Short Time
IDD	Iodine Deficiency Disease
IGAD	Inter-Governmental Authority on Development
LP-s	Lactoperoxidase system
ppm	Parts per million
SADC	Southern African Development Community
UHT	Ultra-high temperature (sterilization) / Ultra heat treated (milk)
WHO	World Health Organization

Executive Summary

This technical meeting was jointly organised by the Animal Production and the Food Quality and Standards Services of the Food and Agriculture Organization of the United Nations (FAO), in cooperation with the Department of Food Safety, Zoonoses and Foodborne Disease of the World Health Organization (WHO) to obtain the best available scientific advice on issues related to the use of the lactoperoxidase system (LP-s) in raw milk preservation.

After reviewing the available scientific information (References, Appendix A and B), the technical meeting concluded that the LP-s 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. The LP-s is particularly suitable for application in situations where technical, economical and/or practical reasons do not allow the use of cooling facilities for maintaining the quality of raw milk. Use of the LP-s does not preclude or replace the need for the pasteurization of raw milk to improve safety for human consumption.

Post harvest losses are a major issue in dairying in developing countries. Smallholder dairy farmers could increase their participation in worldwide milk production, processing and marketing if they could reduce their losses using any approved milk preservation method. Refrigeration is the preferred means of milk preservation but does require high capital investment and can incur high running and maintenance costs. The LP-s provides a cost effective method to increase the availability of milk that contributes to income generation, household food security and nutrition in developing countries.

The LP-s elicits antimicrobial activity against a wide variety of milk spoilage and pathogenic microorganisms including bacteria, HIV-1 virus, moulds, yeasts, mycoplasma and protozoa. Furthermore, the LP-s does not promote the growth of pathogenic microorganisms after completion of the bacteriostatic effect[1]. The activated LP-s is effective in raw milk of different species, the overall activity being primarily bacteriostatic[2], depending on the initial total bacterial load, species and strains of contaminating bacteria and the temperature of milk.

[1]Under laboratory conditions.

[2]The LP-s is classified as a 'microbiostatic' in the Codex Code of Hygienic Practice for Milk and Milk Products (CAC/RCP/57 – 2004) (CAC, 2004b).

Observations from laboratory and field studies indicate that the LP-s does not induce any significant adverse effects on the chemical, physical or sensory characteristics of raw milk and processed dairy products. Under practical conditions the activated LP-s cannot be used to disguise milk of poor microbiological quality.

None of the components of the LP-s presents a significant toxicological risk to public health at the levels proposed. Where iodine deficiency is common, public health measures to rectify the iodine deficiency are needed whether or not the LP-s is used.

In adopting the "Guidelines for the preservation of raw milk by use of the lactoperoxidase system" in 1991, the Codex Alimentarius Commission agreed to emphasise that the LP-s should not be used for products intended for international trade. This provision is considered a major obstacle to the adoption of the system, limiting both regional and international trade in LP-s treated milk and dairy products.

Based on the available data and an assessment thereof, the technical meeting considered the LP-s to be a safe method of raw milk preservation when implemented according to established Codex guidelines. The meeting concluded that this report provides a scientific basis for Codex to reconsider the provision related to the limitation on the international trade of LP-s treated milk and dairy products.

Recommendations

In making its recommendations, the meeting reiterated the safety of the Lactoperoxidase system of raw milk preservation when used according to the existing guidelines (CAC, 1991b), recommending its use in situations when technical, economical and/or practical reasons do not allow the use of cooling facilities. Based on its deliberations the following specific recommendations were made.

TO CODEX

Consider expanding the guideline for the use of this system with regard to temperature of application of the LP-s to also include the temperature range from 31°C to 35°C for 4–7 hours and down to 4°C for 5–6 days.

Develop milk and dairy product standards that can be easily adopted at regional or national level through the encouragement and support of active participation of a representative range of country members in the development of standards.

Remove the current provision regarding the restriction on the use of LP-s in milk or dairy products intended for international trade as the meeting found no scientific or technical basis or economic justification for the provision.

TO MEMBER COUNTRIES, FAO, WHO, CODEX, NGO'S AND THE DAIRY INDUSTRY

Acknowledge the LP-s as an effective and feasible method of raw milk preservation that does not display a negative impact on the further processing of milk.

Owing to its bacteriostatic effect, give consideration to the application of the LP-s as part of a programme to improve milk hygiene and safety along the milk chain.

Consider the application of the LP-s to complement cooling in order to extend the keeping quality of raw milk and halt proliferation of milk spoilage and pathogenic microorganisms.

Use the LP-s to improve the quality of processed products based on its proven bacteriostatic effect from milk collection to final processing and in particular to extend milk collection distances in developing countries, thereby increasing the amount of milk available for marketing. This can have significant direct benefits for both milk producers and consumers.

Recognise that the use of the LP-s is an economically viable option (either standalone or in combination with refrigeration) to significantly reduce milk losses and increase milk availability.

In addition to recommendations specific to the use of the LP-s a number of other related issues were discussed, based on which the technical meeting made the following recommendations.

Promote the consumption of milk as a valuable source of human nutrition contributing to healthy development and growth.

Promote the contribution of small-scale dairying to household nutrition, food security, and poverty alleviation.

Implement measures to rectify iodine deficiency in recognised IDD areas accompanied by appropriate monitoring of its prevalence. Milk can also be a valuable source of iodine, providing there is adequate iodine in the diet of the milk-producing animals.

1. Introduction

This technical meeting was jointly organised by the Animal Production and the Food Standards and Quality Services of the Food and Agriculture Organization of the United Nations (FAO), in cooperation with the Department of Food Safety, Zoonoses and Foodborne Disease of the World Health Organization (WHO) to get the best available scientific advice on issues related to the Lactoperoxidase system (LP-s). The LP-s consists of the addition of sodium thiocyanate and hydrogen peroxide to reactivate the existing lactoperoxidase enzyme in milk that maintains the initial quality of the milk without refrigeration until the milk can be processed or pasteurized.

FAO and WHO recognise the important role smallholder dairy producers play in supplying milk and dairy products to markets in developing countries. Their continued participation in these markets is encouraged. Milk is an important commodity that contributes to household nutrition and health, and can also provide an income. Therefore, approaches for enhancing the availability of safe milk and dairy products are important for the continued improvement of household nutrition and health.

This meeting was part of the FAO/WHO activities on the provision of scientific advice to Codex and to their member countries. The Codex guidelines (CAC /GL 13 – 1991(CAC, 1991b)) for the preservation of raw milk by use of the LP-s were adopted in 1991 at which time the Codex Alimentarius Commission (CAC) also "agreed to emphasise that the lactoperoxidase system not be used for products intended for international trade" (CAC, 1991a). Since then many member countries have raised concerns over this provision. In this regard, FAO and WHO have been asked to provide scientific advice based on comprehensive and relevant information in order to support appropriate decision-making within the Codex system on the use of the LP-s (CAC, 2004a).

Experts from five regions – Africa, Asia, Europe, North and Latin America, and the Caribbean – participated in the meeting in their independent professional capacities and not as representatives of their governments, employers, or institutions. The meeting was supported by a number of submitted papers following an open call for information and data from member countries on issues relating to the LP-s. In particular, issues related to microbiological effects and performance, human health and nutrition, processing and technology, and economic value and trade were addressed. These documents, as listed in Appendix A, were distributed to the experts prior to the meeting. Additional materials consulted and provided by participants during the meeting are included in the Reference section and Appendix B of this report.

1.1 BACKGROUND

This meeting was part of the FAO/WHO activities on the provision of scientific advice to Codex and to their member countries. The Codex guidelines (CAC /GL 13 – 1991(CAC, 1991b)) for the preservation of raw milk by use of the LP-s were adopted in 1991 at which time the Codex Alimentarius Commission (CAC) also "agreed to emphasise that the lactoperoxidase system not be used for products intended for international trade" (CAC, 1991a). Since then many member countries have raised concerns over this provision. In this regard, FAO and WHO have been asked to provide scientific advice based on comprehensive and relevant information in order to support appropriate decision-making within the Codex system on the use of the LP-s (CAC, 2004a).

Lactoperoxidase is an enzyme that is naturally present in milk. One of its unique biological functions is a bacteriostatic effect in the presence of hydrogen peroxide and thiocyanate. Both of these substances are naturally present in milk in varying concentrations. The method of activating the LP-s in milk is to add about 10 ppm (parts per million) of thiocyanate (preferably in powder form) to the raw milk to increase the overall level to 15 ppm (around 5 ppm is naturally present). The solution is thoroughly mixed for 30 seconds and then an equimolar amount (8.5 ppm) of hydrogen peroxide is added (generally in the form of a granulated sodium carbonate peroxyhydrate). The activation of the lactoperoxidase has a bacteriostatic effect on the raw milk and effectively extends the shelf life of raw milk for 7–8 hours under ambient temperatures of around 30°C or longer at lower temperatures. This allows adequate time for the milk to be transported from the collection point to a processing centre without refrigeration.

There are several ways in which the spoilage of milk may be controlled, including refrigeration, heat treatment (pasteurization in bulk or in pouch), microfiltration (with or without pasteurization), bactofugation, high-pressure treatment and use of chemical preservatives (including salting at levels of 3–12%). Some of these procedures require expensive equipment and are not widely applicable, particularly in small-scale dairy production and processing systems in developing countries where up to 80% of the milk produced may enter the informal market.

The FAO Global Lactoperoxidase Experts Group (GLP) was set up in July 1998. The main objective of this group was to promote the LP-s and carry out demonstrations in specific regions in the world where refrigeration is difficult. The partners involved in this group were the Lund University of Sweden, WHO, the International Dairy Federation, and FAO, with support from the Governments of Sweden, France, Hungary and Ireland. The strategy of the GLP was to inform countries and assess their interest in these issues, to identify regional partner institutions, national institutions and experts, conduct national training and demonstrations in collaboration with the relevant ministries and follow-up through national experts and governments. The outputs from the GLP included posters and manuals on the use of the LP-s in English, French and Spanish, the printing and distribution of Field Manuals, the implementation of training and demonstrations in 35 countries, annual meetings and the Bushmilk *(Lait de brousse)* programme in West Africa.

Codex adopted the "Guidelines for the preservation of raw milk by use of the lactoperoxidase system" in 1991 (CAC, 1991a, b). Issues concerning the LP-s of raw milk preservation have been raised in numerous Codex meetings, most recently during the meeting of the Codex Alimentarius Commission in Geneva in 2004 (CAC, 2004a). Issues related to the guidelines have also been raised as a concern by numerous FAO member countries.

In 2002, the GLP requested that the Codex Committee on Milk and Milk Products (CCMMP) consider amendments to the guidelines (CAC, 2002a). Highlighting the need for a scientific basis for any amendments, the committee referred the issue to the Codex Executive Committee later in 2002, which agreed that this might be of particular interest to developing countries and invited Regional Committees to consider the issues (CAC, 2002b). It was recognised that all relevant health aspects of this complex issue should be considered to ensure that any revision of the guidelines would be based on sound science and risk analysis.

In 2002, the Codex Coordinating Committee for Africa supported these decisions and maintained that until uncertainties related to the process were resolved the provisions on the use of this system should be maintained (CAC, 2002c). The Codex Coordinating Committee for North America and South West Pacific (CCNASWP) in 2002 also recommended that further reviews by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) of the chemical and microbiological safety of the LP-s should be undertaken before revising the guidelines (CAC, 2002d). In 2003, the Codex Committee on Food Hygiene (CCFH) concluded that the current provision excluding the use of the lactoperoxidase system for products intended for international trade should continue to be applied and that there was no need for the revision of the existing Guidelines in the framework of Codex or a JECFA review (CAC, 2003).

The issue was raised again in the CCFH in 2004 when the committee was informed that new data were being generated. It was also discussed at the 27th session of the Codex Alimentarius in 2004 in the course of the adoption of the Draft Code of Practice for Milk and Milk Products, during which the following text was added to the code, "The use of the lactoperoxidase system for milk and milk products in international trade will be re-examined by the Committee on Food Hygiene (CCFH) after completion of an expert review by FAO and WHO of available data and considering the FAO Lactoperoxidase Expert Group report about potential risks and benefits of lactoperoxidase system. CCFH will then review the issue in 2006" (CAC, 2004a).

1.2 SCOPE AND PURPOSE OF THE TECHNICAL MEETING

The current meeting was implemented to respond to member country concerns and to provide scientific advice to the next session of the CCFH in 2006 on the benefits and possible risks associated with the LP-s for raw milk preservation and any dairy products derived from treated milk.

The objective of the technical meeting was to determine the benefits (economic and nutritional) and the level of health risks, if any, posed by the application of the LP-s, advise on the safety of the LP-s treated milk and derived milk products, and to address the issue of the limitation on the use of LP-s treated milk or derived products intended for international trade.

The group agreed to discuss these issues under the following four headings and a chairperson and rapporteur was assigned for each of the four subject areas.

1. The microbiological effects and performance of the LP-s
Chairperson: C. Michiels
Rapporteur: H. Korhonen

2. The effects of LP-s treated milk and dairy products on human health and nutrition
Chairperson: J. Vanderveen
Rapporteur: R. Walker

3. Milk processing, technology and preservation
Chairperson: J. P. Ramet
Rapporteur: A. Grandison

4. Economic value and trade of LP-s treated milk and dairy products
Chairperson: H. G. Muriuki
Rapporteur: O. C. Emata

This report summarises the deliberations, findings and conclusions of the meeting.

2. Microbiological Effects and Performance of the Lactoperoxidase System

The effectiveness of the LP-s in maintaining the hygienic quality of raw milk for a limited period of time has been established in many experimental and field studies conducted in different geographical regions. The method can be applied to preserve raw milk from different species. The effectiveness depends on the initial amount and type of microbiological contamination and the temperature of milk during the treatment period. The LP-s exerts primarily a bacteriostatic effect in raw milk. Experimental data and experience from practice indicate that the LP-s can be applied beyond the temperature limits (15–30°C) referred to in the 1991 Codex guidelines (CAC, 1991b). At the lower end of the temperature scale, several studies indicate that activation of the LP-s can delay growth of psychrotrophic milk bacteria and thus delay milk spoilage for several days compared to what can be achieved with refrigeration alone.

It is important to emphasise that the purpose of the use of the LP-s is not to render milk safer for consumption but to preserve its initial quality. Good hygienic practices in milk production are critical to the efficacy of the LP-s and to the microbiological quality of the milk. The safety of milk is only achieved through a combination of good hygienic practices and heat treatment of milk, independent of the LP-s. This effectiveness of the LP-s under various conditions and against a range of microorganisms is addressed below.

2.1 EFFECTIVENESS OF THE LACTOPEROXIDASE SYSTEM FOR PREVENTING SPOILAGE OF RAW MILK

a) Effectiveness under conditions as specified in the Codex guidelines

The Codex guidelines focus on the application of the LP-s for preventing spoilage of raw milk (bovine and buffalo) during collection and transportation to a dairy processing plant, under conditions where adequate refrigeration is not feasible. The guideline is based on a number of scientific papers from the late 1970s elucidating the working principles of the method and providing proof of concept (Björck, Claesson and Schulthess, 1979; Reiter et al., 1976; Björck, 1978).

Since the adoption of the Codex guidelines, a substantial amount of data on the effectiveness of the LP-s has accumulated, not only from laboratory and field studies, but also from experience with the large-scale adoption of the system in commercial milk production in some countries. During the meeting, summary reports showing results from many

countries, for example Cuba, Colombia, Peru, Venezuela, Cameroon, Kenya, Uganda and Pakistan, covering a wide range of different production conditions, were presented and have been reviewed (Björck, Claesson and Schulthess, 1979; Bibi and Bachmann, 1990; Ponce et al., 2005; Albujar, Ludena and Castillo, 2004; Siirtola, 2005; Fonteh, Grandison and Lewis, 2005). Overall, these data confirm the effectiveness of the LP-s for preventing spoilage of non-refrigerated raw milk within the framework defined in the Codex guidelines, i.e.:

- The principles of good hygienic practice in milk production must be respected in order to guarantee a good initial microbiological quality of the raw milk (see below)

- The inhibitory effect of the treatment is dependent on the storage temperature of LP-s treated milk as follows (Table 1):

Table 1: Extension of milk keeping quality by the LP-s at different temperatures

Temperature (°C)	Time (hours)	Reference
31-35	4-7	Ponce et al., 2005
30	7-8	CAC,1991b
25	11-12	CAC,1991b
20	16-17	CAC,1991b
15	24-26	CAC,1991b
4	5-6 days	Zapico et al., 1995; Lin and Chow, 2000

It should be emphasised that these spoilage delay times should be considered indicative, because they are affected to a great extent by the initial bacterial load (see below).

b) Effectiveness under different ambient conditions

The temperature dependence of the effectiveness of the LP-s as shown above, and as already specified in the original CAC guidelines (CAC, 1991b), illustrates that with respect to prevention of spoilage of raw milk, the LP-s can be complementary to refrigeration. In other words, it can compensate for a lack of refrigeration whenever the latter cannot be supplied. However, the efficacy of the LP-s persists for a limited period of time, which decreases as the ambient temperature increases. This temperature dependence of the effectiveness of the LP-s was defined only in a range between 15 and 30°C in the original Codex guidelines. However, milk storage temperatures may exceed 30°C during daytime, and may fall below 15°C during night-time in some regions without refrigeration facilities. Therefore, the effectiveness of the LP-s at temperatures outside this range is a relevant issue.

Temperature is one of the most important factors influencing microbial growth. The role of refrigeration and the cold chain in maintaining the quality and safety of both raw and pasteurized milk is well recognised. Many bacteria are mesophilic, growing best at

temperatures of 30°C to 40°C. However, psychrotrophic and psychrophilic bacteria can grow at low temperatures, with some strains capable of surviving and growing at temperatures down to 0°C. *Listeria monocytogenes* is an example of a pathogenic bacterium that can grow at very low temperatures. However, in products such as milk that have a diverse microflora, it would normally be outgrown by the psychrotrophic spoilage bacteria, such as members of the genera, *Pseudomonas, Bacillus* and *Micrococcus.*

Some recent field studies that have been carried out with raw milk treated by the LP-s and stored at 30–35°C showed a consistent inhibition of microbial growth for 4–7 hours (Ponce *et al.*, 2005).

Effectiveness of the LP-s may also be relevant to microbial quality and safety issues in relation to extended storage of raw milk under refrigerated conditions. Current issues of concern with regard to low temperature storage include the formation of heat stable proteases by psychrotrophic *Pseudomonas* spp. and the outgrowth of psychrotrophic pathogens such as *Listeria monocytogenes* and some *Bacillus cereus* spp.. At this end of the temperature scale, several studies indicate that activation of the LP-s can delay growth of psychrotrophic milk bacteria and thus delay milk spoilage for several days compared to what can be achieved with refrigeration alone. For example, studies in Taiwan indicated a six-day extension of the spoilage-free storage period of raw milk at 4°C upon activation of the LP-s (Lin and Chow, 2000). Another study showed that the LP-s prevented the growth of psychrotrophic *Pseudomonas fluorescens* for five days at 4°C and for three days at 8°C (Zapico *et al.*, 1995). A summary table comparing LP-s, refrigeration and the combination of LP-s with refrigeration is included as Appendix C.

c) Effectiveness in milk of different species (bovine, buffalo, sheep, goat, camel)

The lactoperoxidase enzyme is present in the milk of all mammals. Although there are variations at the species and even at the individual animal level (Fonteh, Grandison and Lewis, 2002), the enzyme levels in the milks that are used for human consumption are not believed to be a limiting factor for the effectiveness of the LP-s. In general, the available studies show that the time/temperature combination as outlined for cow and buffalo milk are also applicable to goat and sheep milk. In camel milk, the activation of the LP-s may induce a longer-lasting bacteriostatic effect than in cow's milk due to the presence of higher levels of other indigenous antimicrobial components (Ramet, 2001). Less information is available for milk from other species.

d) Effectiveness in relation to principles of hygienic milk production

The Codex guidelines state that, *"Due to the mainly bacteriostatic effect of the system it is not possible to disguise poor quality milk, which originally contained a high bacterial population, by applying this method", and, "The use of the LP-s does not exclude the necessity of pasteurization of milk before human consumption. Neither does it exclude*

the normal precautions and handling routines applied to ensure a high hygienic standard of the raw milk" (CAC, 1991b).

Microbiological studies conducted over the last 10 to 15 years support this view. Invariably, the antibacterial efficacy of the LP-s is found to be inversely correlated to bacterial cell density. The antibacterial efficacy of the LP-s is low at high bacterial concentrations, primarily bacteriostatic at intermediate concentrations and primarily bactericidal at low concentrations. This follows from both laboratory observations with pure cultures of pathogenic or spoilage bacteria suspended in buffer or broth (El-Shenawy, Garcia and Marth, 1990; Garcia-Graells *et al.*, 2003), and from field studies in milk with its natural mixed microflora (Ponce, 2005; Albujar, Ludena and Castillo, 2005). Consequently, safeguarding a high bacteriological milk quality before application of the system by adopting good hygienic practices is critical to its efficacy. In this respect, the use of the LP-s for preserving the quality of the milk before pasteurization does not differ from use of refrigeration for the same purpose. It is important to emphasise that the purpose of both methods is to prevent (microbiological) deterioration of the milk after milking and before pasteurization, not to render the milk safer for consumption, which is achieved by subsequent pasteurization of milk.

2.2 EFFECTIVENESS OF THE LACTOPEROXIDASE SYSTEM AGAINST PATHOGENIC MICROORGANISMS

The antimicrobial activity of the LP-s in milk, whey and synthetic media has been demonstrated against a wide range of microorganisms, including bacteria, HIV-1 virus, moulds, yeasts, mycoplasma and protozoa (for reviews see Korhonen, 1980; Reiter and Härnulv, 1984; IDF, 1991; Wolfson and Sumner, 1993; Stadhouders and Beumer, 1994; de Wit and van Hooijdonk, 1996; van Hooijdonk, Kussendrager and Steijns, 2000; Seifu, Buys and Donkin, 2005). These microorganisms cover non-pathogenic starter cultures and spoilage bacteria as well as pathogenic organisms that cause gastrointestinal infections in humans and udder infections in cows. However, considerable differences have been found in the sensitivity of different bacteria to the LP-s. Depending on the bacterial species or even the strain of the organism, the effect can be either bactericidal or bacteriostatic even under identical conditions. The LP-s has been found to be less effective against some non-pathogenic streptococci and lactococci.

The variations in sensitivity between strains may be explained by different cell wall structures and inhibitory compounds generated by the organisms concerned. Lactic acid bacteria, for example, are deficient in the catalase enzyme, and many species metabolically produce H_2O_2, which is accumulated in the growth medium. This H_2O_2 can activate the LP-s and lead to the self-inhibition of bacterial growth. Many dairy cultures are sensitive to the LP-s, and while some reports indicate interference with the fermentation processes (Wright and Tramer, 1958; De Valdez, Bibi and Bachmann, 1988; Seifu, Buys and Donkin, 2003), the impact is not consistent. This issue is also addressed in section 4.4. Most Gram-negative bacteria possess the catalase enzyme, which decomposes any

generated H_2O_2. These bacteria, therefore, are not self-inhibited in milk through the LP-s and, to activate the system, H_2O_2 has to be supplied from an exogenous source, e.g. by the addition of sodium percarbonate. Under such conditions Gram-negative pathogenic and spoilage bacteria can be killed or their growth arrested for a certain period of time (Reiter *et al.,* 1976; Sandholm *et al.*, 1988; Dionysius, Grieve and Vos, 1992).

A number of studies on the impact of the LP-s on some of the most common milk-borne pathogens and other microorganisms causing infections in humans and domestic animals have been undertaken. Some of those on common milk-borne pathogens, namely *Escherichia coli, Salmonella spp., Campylobacter spp., Staphylococcus aureus, Listeria monocytogenes, Yersinia enterocolitica* and *Brucella melitensis* are summarised in Table 2. In various experimental studies, the bacteriostatic or bactericidal effect of the LP-s has been demonstrated against several other human pathogenic microorganisms, such as *Streptococcus mutans* (Carlsson, Iwami and Yamada, 1983), *Aeromonas hydrophila* (Santos *et al.*, 1995), *Candida albicans* (Lenander-Lumikari, 1992) and *Helicobacter pylori* (Shin *et al.,* 2002). Also, the LP-s has been shown to inhibit the reverse transcriptase enzymatic activity of HIV-1 virus (Wang, Ye and Ng, 2000). Furthermore, a recent study by Armenteros *et al.*, (2005) has shown that the activation of the LP-s in raw milk does not exacerbate the presence of human pathogens including *E. coli* O157: H7, *L. monocytogenes, S. aureus and* S. Typhimurium when introduced into raw milk under laboratory conditions.

The LP-s is considered as one of the body's natural defence mechanisms against microbial infections. Increased concentrations of lactoperoxidase and thiocyanate ions are found in milk from mastitic cows as compared to milk from healthy animals. In general, the same applies to other major antimicrobial factors occurring in milk, e.g. immunoglobulins, lactoferrin, lysozyme and phagocytic cells (Korhonen *et al.*, 1977; Reiter, 1978; Reiter, 1985; Reiter and Perraudin, 1991; Korhonen, 2002). The LP-s has been shown to be bactericidal or bacteriostatic in vitro against many microorganisms that cause udder infections, e.g. *E. coli, S. aureus, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis and Pseudomonas aeruginosa* (Mickelson, 1966; Reiter *et al.,* 1976; Marshall, Cole and Bramley, 1986; Sandholm *et al.*, 1988). Many of these bacteria also pose a potential risk to human health. There is some experimental data to show that the LP-s in mastitic milk is not as effective as in milk from healthy cows because of a higher concentration of reductive agents and higher catalase enzyme activity present in mastitic milk (Sandholm *et al.,* 1988). No studies have so far been reported on the antibacterial activity of the LP-s against antibiotic-resistant mastitis organisms or coagulase-negative staphylococci. These organisms are frequently isolated from mastitic udders.

Table 2: Summary of studies on the impact of the LP-s on some common milk-borne pathogens

Pathogen	Effect of LP-s	Demonstrated in	Reference
Escherichia coli, including *E. coli* O157:H7	Bactericidal	Raw cow milk, buffer solution and synthetic medium Infected calves and piglets	Reiter *et al.*, 1976; Reiter, Marshall and Philips, 1980; Earnshaw *et al.*, 1990; Farrag, El-Gazzar and Marth, 1992a; Grieve, Dionysius and Vos, 1992; Zapico *et al.*, 1995; Kangumba, Venter and Coetzer, 1997; Heuvelink *et al.*, 1998; Bosch, van Doormen and De Vries, 2000; Seifu, Dunkin and Buys, 2004
	Reduced gastrointestinal colonization rate of coliform bacteria Bacteriostatic	Raw cow, goat and camel milk, culture medium and infant formula	
Salmonella Typhimurium	Bactericidal and bacteriostatic (dependent on number of organisms)	Raw milk	Reiter *et al.*, 1976; Purdy *et al.*, 1983; Earnshaw *et al.*, 1990; Pitt, Harden and Hull, 2000
Salmonella typhi, other *Salmonella* spp.	Bactericidal	Culture medium, infant formula and fresh cheese	
Campylobacter jejuni (various strains)	Bactericidal	Cow Milk	Borch *et al.*, 1989; Beumer *et al.*, 1985
Staphylococcus aureus (several strains)	Bactericidal and bacteriostatic	Cow, goat and camel milk	Kamau, Doores and Pruitt, 1990; El-Agamy *et al.*, 1992; Kangumba, Venter and Coetzer, 1997; Pitt, Harden and Hull, 2000; Seifu, Donkin and Buys, 2004
Listeria monocytogenes (several strains)	Bactericidal and bacteriostatic (activity depending on temperature, length of incubation and strain)	Raw cow and goat milk, UHT milk, soft cheese and in synthetic medium	Dennis and Ramet, 1989; Siragusa and Johnson, 1989; Bibi and Bachmann, 1990; El-Shenawy, Garcia and Marth, 1990; Gaya, Medina and Nuñez, 1991; Zapico *et al.*, 1993; Pitt, Harden and Hull, 1999; Seifu, Donkin and Buys, 2004; Gay and Amgar, 2005
Yersinia enterocolitica	Bactericidal	Cow milk	Beumer *et al.*, 1985; Farrag, El-Gazzar and Marth, 1992b
Brucella melitensis	Bactericidal	Goat milk	Seifu, Donkin and Buys, 2004;

2.3 POSSIBLE CONSEQUENCES OF THE LONG-TERM USE OF THE LACTOPEROXIDASE SYSTEM ON ITS ANTIMICROBIAL EFFICACY

The issue of whether long-term use of the LP-s would result in any microbiological risks, e.g. development of LP-s resistant, antibiotic-resistant or toxin-producing bacteria was considered.

Some studies show that the efficacy of the LP-s could be interfered with by residues in milk of certain antibiotics used in the treatment of mastitis (Ali-Vehmas, Vikerpuur and Sandholm, 1994). Mutants of *Escherichia coli* with increased tolerance to the LP-s have recently been isolated in the laboratory and characterised (De Spiegeleer *et al.,* 2005). For one category of such mutants (*waaQ* and *waaO*), LP-s tolerance was linked to a deficiency in the outer core polysaccharide of the lipopolysaccharides, which causes a reduced permeability of the outer membrane for the hypothiocyanate anion (OSCN-) due to a reduced porin content in the outer membrane. This type of mutation also causes a slightly elevated resistance to some penicillins (Nikaido, 2003). However, LP-s tolerant mutants have never been isolated from LP-s treated milk, which may be due to a reduced fitness under these conditions. For example, the *waaQ* mutation mentioned above causes a so-called rough phenotype, which is also associated with enhanced sensitivity to lactoferrin and lysozyme, two other important antimicrobial factors in milk. Thus, the available data indicate that adoption of the LP-s is not likely to stimulate the development of resistance to the LP-s itself or antibiotic-resistant microorganisms. However, as with all antimicrobial systems and due the ability of microorganisms to adapt the meeting considered that ongoing monitoring and research in this area is warranted.

2.4 CONCLUSIONS AND RECOMMENDATIONS

The LP-s elicits antimicrobial activity against a wide variety of milk spoilage and pathogenic microorganisms including bacteria, viruses, moulds, yeasts, mycoplasma and protozoa. The overall activity is primarily bacteriostatic[3], depending on the initial total bacterial load, species and strains of contaminating bacteria and the temperature of milk. While its effectiveness against well-known milk spoilage and pathogenic microorganisms is well established, further studies would be useful on the efficacy of the LP-s against milk-borne viruses and emerging pathogenic microorganisms.

The activated LP-s is effective in raw milk of different species and available studies also indicate that the same time-temperature as outlined in the Codex guidelines (CAC, 1991b) can be applied to goat and sheep milk.

The LP-s does not promote the growth of pathogenic microorganisms after completion

[3] The LP-s is classified as a 'microbiostatic' in the Codex Code of Hygienic Practice for Milk and Milk Products(CAC/RCP/57 – 2004) (CAC, 2004b).

of the bacteriostatic effect and there is no evidence to show that the long-term use of the LP-s would lead to any such microbiological risks, e.g. development or accumulation of toxin-producing bacteria.

Under practical conditions the activated LP-s cannot be used to disguise poor microbiological quality of milk. Good hygienic practices in milk production are critical to the efficacy of the LP-s.

LP-s is effective in refrigerated raw milk. Experimental and field studies have demonstrated that the activated LP-s is effective in prolonging the keeping quality of raw milk both for up to 5–6 days in refrigerated milk (+4°C) and up to 4–7 hours at high ambient temperatures (from 31 to 35°C).

The application of the LP-s is not likely to stimulate the development of resistance to the LP-s itself or other antimicrobial agents but due to the dynamic nature of microorganisms ongoing monitoring of the situation would be reasonable.

Based on the above the meeting recommended that:

- When refrigeration is not technically feasible or economically viable the LP-s be applied to raw milk to halt proliferation of milk spoilage and pathogenic microorganisms.

- The application of the LP-s should be considered as part of a programme to improve milk hygiene and safety along the milk chain, owing to its bacteriostatic effect.

- Consideration be given to the application of the LP-s to complement cooling in order to extend the keeping quality of raw milk.

- Codex consider expanding the guideline for the application of the LP-s with regard to temperature of application to also include the temperature range from 31 to 35°C for 4–7 hours and down to 4°C for 5–6 days.

- Monitoring for the development of resistance be undertaken to detect the development of any resistant microorganisms.

3. Human Health and Nutrition

Milk has an important nutritional role in the diet, particularly for growing children, throughout the world and not just in developing countries. It represents a major source of protein, calcium, phosphorus, magnesium, and fat-soluble vitamins and may make a significant contribution to dietary intakes of some other vitamins and minerals including iodine. Milk can also be a useful vehicle for supplementation of nutrients such as vitamins A and D (WHO, in press). Lactose in milk is involved in regulating osmotic pressure but an additional role in facilitating calcium absorption in infants has been suggested (Abrams, Griffin and Davila, 2002; Garrow, James and Ralph, 2000).

There is a negative correlation between milk consumption and morbidity and mortality from childhood diseases and in this respect the provision of school milk programmes has been effective in improving childhood health and nutritional status (Scrimshaw and San Giovanni, 1997).

While the condition of lactose intolerance may limit the amounts of milk that can be consumed without adverse effects by some individuals/populations, up to one cup of milk (approx. 200ml) is generally tolerated. Furthermore, lactose serves as a substrate in lactic fermented milk products, leading to a reduction in the levels in such products and yeast fermentation results in hydrolysis of lactose by microbial Đ-galactosidase. Considering the important role of milk in human nutrition and health this section addresses the impact of the application of the LP-s for raw milk preservation from a public health and nutrition perspective.

3.1 THE LACTOPEROXIDASE SYSTEM IN CONTEXT

The LP-s differs uniquely from other preservation systems in that it is a natural biological protective system in the biology of animals. It functions as a protective antimicrobial mechanism in mucosal tissue, including in the oral cavity and lung (Tenovuo, 2002; Geiszt *et al.,* 2003). In this regard, the LP-s does not introduce substances into milk that are not normal human metabolites.

The LP-s can be applied to reduce spoilage of milk where refrigeration is not immediately available. However, the use of LP-s is not exclusive and may be combined with other procedures (e.g. refrigeration) to reduce losses of milk both in the formal and informal markets. The safety evaluation of the use of the LP-s in milk by JECFA at its 35th meeting (see below) was restricted by the terms of reference to the application of the system "when refrigeration is virtually impossible", and by the Guidelines drafted by the Joint FAO/WHO Committee of Government Experts on the Code of Principles

concerning Milk and Milk Products. It is recognised that the safety issues concerning the broader application of the LP-s in conjunction with other methods for controlling spoilage, including refrigeration, were not addressed at that time.

3.2 POTENTIAL HEALTH ISSUES ASSOCIATED WITH THE USE OF THE LACTOPEROXIDASE SYSTEM: TOXICOLOGICAL ASPECTS

As noted above, the components or metabolites of the LP-s, namely lactoperoxidase, the thiocyanate ion and hypothiocyanate have been detected in animal and human tissues and secretions, including milk. The levels of hydrogen peroxide introduced into the milk via sodium percarbonate are lower than those previously considered acceptable by the 24th meeting of the JECFA (WHO, 1980) and are, therefore, not of concern.

The use of the LP-s does not require the addition of further lactoperoxidase above the levels of the enzyme occurring in raw milk. As there is no change to the enzyme concentrations naturally present in milk, this component is not considered of toxicological significance.

Hypothiocyanate has been detected in human saliva (Thomas, Bates and Jefferson, 1980) and has a very short half-life in milk, so that residual levels in milk treated with the LP-s do not pose a toxicological risk. The breakdown products are considered innocuous.

In the earlier evaluation, at its 35th meeting in 1990 the JECFA concluded that *"when used according to the draft guidelines, the lactoperoxidase system does not present a toxicological hazard and, furthermore, that the system should be used in preference to hydrogen peroxide alone for the preservation of raw milk, though only where absolutely necessary i.e. in the absence of adequate refrigeration facilities"*. Very few new data on the toxicology of thiocyanate have become available since the previous JECFA evaluation.

The present group examined the potential toxic effect of thiocyanate, which was considered to interfere with iodine metabolism and uptake by the thyroid (WHO, 1990). The mode of action of the goitrogenic effect is via competitive inhibition of iodine and tyrosine oxidation leading to lower levels of thyroxine (T4) and inhibition of uptake by the thyroid. However, this effect occurs at relatively high plasma thiocyanate concentrations (60–80 micromolars or 4.8-6.4 milligram/litre) whereas at lower levels (0.5–1.0 µmolar) there is a stimulatory effect by interacting with thyroid peroxidase (Green, 1978).

At high plasma thiocyanate concentrations there is an increased excretion of iodine and a reduced iodine uptake by the thyroid gland, resulting in a low thiocyanate/iodine (SCN/I) excretion ratio. The value of the threshold level for this ratio seems to be three (Delange and Ahluwalia, 1983) after which endemic goitre appears. This phenomenon can occur only when the iodine intake is below about 100 micrograms per day. At SCN/I ratios of lower than two there is a risk to cognitive function and development (Erman *et*

al., 1983). A low ratio leads to abnormal levels of the thyroid stimulating hormone (TSH) and low thyroxine (T4). Ayangade, Oyelola and Oke (1982) found that in pregnant women the thiocyanate level of the cord blood was proportional to the maternal serum thiocyanate level, indicating that thiocyanate can cross the placental barrier and affect the foetus. However, there is very little thiocyanate in breast milk indicating that the mammary gland does not concentrate thiocyanate and so breast-fed infants are not affected.

In this context, in clinical studies on sodium thiocyanate in milk, negative effects on iodine metabolism were only observed at concentrations of 200–400 milligrams/litre (Vilkki and Piironen, 1962). Furthermore, in studies in normal euthyroid individuals no significant effects on thyroid function (T4, T3, TSH) resulted from consumption of 8 milligrams of thiocyanate in milk daily for 12 weeks (Dahlberg *et al*., 1984) although serum and urinary levels increased. Conversely, the group with a (presumed) daily consumption of milk containing about 45 milligrams/litre had higher serum levels of T4 and lower T3 and TSH levels than a control group (Banerjee *et al*., 1997). It should be noted that this last study was published only as a short communication and the level of reporting did not allow the group to conduct a critical evaluation.

From the foregoing it can be concluded that the groups likely to be at highest risk from thiocyanate exposure are iodine-deficient subjects. However, in one study in which iodine-deficient adults were given milk containing 19 milligrams thiocyanate/litre (controls 3.6 milligrams/litre) leading to an additional daily intake of 4.75 milligrams, there was no apparent effect on thyroid function (Dahlberg *et al*., 1985). The milk used in this study contained iodine at a concentration of 100 micrograms/litre.

There were no experimental data available on the effects of dietary thiocyanate on reproductive function or on the genotoxicity of thiocyanate. Plasma thiocyanate concentrations can reach 100 milligrams/litre during sodium nitroprusside therapy, but toxicity often occurs at concentrations above 120 milligrams/litre. Plasma concentrations in the order of 200 milligrams/litre have been reported in fatalities.

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/litre in drinking water. The results of this study led to the conclusion that sodium thiocyanate is not carcinogenic to rats (Lijinsky and Kovatch, 1989).

The clinical symptoms of overt iodine deficiency during pregnancy as manifested in foetal development and growth of children have been known for more than eighty years. These include stillbirth, abortion and congenital anomalies (Hetzel, 1983; Mastovinovic, 1983). In recent years, research has revealed that iodine deficiencies during pregnancy, even in which overt maternal symptoms are lacking, can have an effect on the growing child, such as hearing deficits (Wang and Yang, 1985).

The normal levels of thiocyanate in milk depend on the levels of thiocyanate and its precursors in the animals' diet, including thioglycosides (glucosinolates) and cyanogenic glycosides. Concentrations have been reported to vary between 2.3 and 35 milligrams/litre in milk from individual cows and to be around 8 milligrams/litre in bulked milk (Ponce *et al.*, 2005). Higher levels occur in colostrum and in mastitis milk. Similar results were obtained for cow milk (6–12 milligrams/litre; mean 8.5 milligrams/litre) and goat milk (6.6–8 milligrams/litre; mean 7 milligrams/litre) (Fonteh, Grandison and Lewis, 2002). When used according to the Codex guidelines, the level of supplementation of sodium thiocyanate in activating the LP-s is 10–15 milligrams/litre so that overall levels in activated bulk milk would be in the order of 20 milligrams/litre, a factor of 10–20 lower than those reported to lead to detected effects on iodine metabolism. A study of the thiocyanate concentrations in milk mixtures under practical conditions of the American tropics indicates that they oscillate between 5.8 and 8.12 milligrams/litre, although the levels in milk of individual cows vary widely, ranging from 2.9 to 34.8 milligrams/litre. That is why the total content of thiocyanate, once the LP-s is activated in a milk mixture, does not surpass the natural maximal concentration in any particular cow milk (Ponce *et al.*, 2005). Evidence of undesirable effects were not observed in the populations consuming milk activated with the LP-s for more than 10 years (Fernandez, Marrero and Capdevila, 2005).

Thiocyanate is found in animal and human tissue and fluids where it is part of the defensive system (e.g. high in colostrums and in milk of cows with mastitis) and is a metabolite of the detoxication process of cyanogenic glycosides. Thiocyanate is also present in foods of plant origin and it is formed in the human or animal body from substances in plants such as glucosinolates (in brassica an average 100 milligrams/kilogram) or cyanogenic glycosides. Thiocyanate is present in raw lima beans (100–3100 milligrams/kilogram), raw cassava tubers (10–462 milligrams/kilogram), raw cassava leaves (68–468 milligrams/kilogram), dried cassava root cortex (2450 milligrams/kilogram), almonds (6.2 milligrams HCN/bitter almond), bamboo shoots tips (8000 milligrams/kilogram), stone fruits and sorghum (2500 milligrams/kilogram) (FAO, 1990). Cyanides readily decompose upon heating, and cooked foods contain little or no cyanide, e.g. cooked cassava tubers had 1-10 milligram/kilogram depending on the cooking method and the initial content. Glucosinolates and glucosinolate breakdown products are hydrophilic, and as much as 63% of the glucosinolate content of a vegetable may leach into the cooking water during boiling (WHO, 1993).

The additional intake of sodium thiocyanate from one cup (200 ml) of LP-s treated milk would correspond to 3 milligrams of sodium thiocyanate which is also present in 30 grams of raw cabbage, 1 gram of raw lima beans or 8 grams of raw cassava tuber. When applying the food supply of the 13 GEMS/Foods regional diets (See Appendix D), exposure to sodium thiocyanate is estimated to be in the range of 2.8 to 9.5 milligrams/day. If all milk were treated with the LP-s the exposure would increase to 5.9 to 21.2 milligrams/day.

The highest potential risk from thiocyanate would arise with infants because of the high need for energy per kilogram bodyweight and the unitary diet. As an example, in a 10 kilogram infant, 500 millilitres of LP-s treated milk would result in 1 milligrams/kilogram body weight of sodium thiocyanate compared to 0.3 milligrams/kilogram body weight from untreated milk. The LD_{50} dose of orally administered sodium thiocyanate in rats, a measure of acute toxicity, is reported to be 764 milligrams/kilogram body weight (FAO/WHO, 1965). Clearly, acute toxicity is not a relevant aspect of exposure through the LP-s treated milk.

In non-smokers, plasma thiocyanate concentrations range from 0.1 to 0.4 milligrams/litre, while in heavy smokers concentrations typically range from 5 to 20 milligrams/litre (WHO, 1995). Thiocyanate is concentrated in other human body fluids, notably saliva and gastric juice, where levels typically range from 10 to 300 milligrams/litre (Björck, Claesson and Schulthess, 1979; Korhonen, 1980; Reiter and Härnulv, 1984; Farrag and Marth, 1992, Food Standards Australia and New Zealand, 2002).

3.3 NUTRITIONAL EFFECTS

The LP-s reduces losses of milk through microbial spoilage and can thus increase the volume of milk available as an important nutritional component of the diet. Although a reduction in folate levels in milk may occur as a result of LP-s treatment, milk is not considered to be a significant dietary source of folate and the overall dietary impact is not considered important.

3.4 EFFECTS ON MILK-BORNE PATHOGENS

Although LP-s may be effective to a limited degree against some pathogens, it should not be considered as an alternative to pasteurization in this regard. The effects on a number of pathogens are dealt with in more detail in section 2. There are no available data on the effects of the LP-s on milk-borne viruses, although some research has been undertaken on the impact of the LP-s on HIV-1 (Wang, Ye and Ng, 2000).

3.5 CONCLUSIONS AND RECOMMENDATIONS

Overall, the meeting considered the LP-s to be a safe method of preventing losses of milk owing to microbial spoilage when used according to the guidelines (and with an extended temperature range as recommended under 2.5) either alone or in combination with other approved procedures.

It was concluded that the advantages of the LP-s mainly result from significantly reduced spoilage losses of milk and thus improved availability of milk as a good nutrient source in the diet and benefiting both milk producers and consumers.

Milk improves health and reduces morbidity and mortality from childhood disease. Therefore, the application of the LP-s could be considered as part of a system to improve public health by increasing the availability and safety of milk.

Based on the available scientific information the meeting concluded that none of the components of the LP-s presents a significant toxicological risk to public health at the levels proposed. Nevertheless, where iodine deficiency is common, public health measures to rectify the iodine deficiency are needed whether or not the LP-s is used.

Based on the assessment, the LP-s is a safe method of raw milk preservation when implemented according to established guidelines (with an extended temperature range as recommended under 2.5); it can reduce milk losses which is a major benefit for both milk producers and consumers.

Based on the above the meeting recommended that:

- The LP-s be considered safe, when used according to the Codex guidelines, for use in situations when technical, economical and/or practical reasons do not allow the use of cooling facilities and that it be applied as part of an integrated programme to improve milk production and quality.

- Milk consumption be promoted because of its value in human nutrition for healthy development and growth.

- Measures to rectify iodine deficiency be implemented in recognised IDD areas accompanied by appropriate monitoring of its prevalence. It was noted that milk could also be a valuable source of iodine, providing there is adequate iodine in the diet of the milk-producing animals.

4. Processing and Technology

Milk is recognised as a highly nutritious food and valuable source of vitamins and minerals. It is, however, highly perishable and has, in its raw state, a relatively short shelf-life. There are numerous processes for prolonging the shelf-life of milk and dairy products and an increasing array of technologies that can be applied to improve the safety and quality of milk.

While refrigeration and heat treatment of raw milk are also highly effective in and widely used for extending shelf-life, more advanced physical treatments are also evolving and being applied such as microfiltration and high pressure processing. The cost of these processes and associated technologies is relatively high as compared to the combination of heating and cooling such as in pasteurization processes (high temperature short time or low temperature long time). Also in many rural areas even the cost of cooling remains prohibitively high. The use of LP-s is not designed to replace adequate heat treatment, which kills harmful bacteria, but has the potential to increase the quality and quantity of raw milk available for further processing into dairy products.

The LP-s is one of the growing families of biostatics that can have beneficial effects in the processing of milk by extending the shelf life and improving the quality of milk collected or preserved. This section reviews the LP-s activation/inactivation and examines potential risks and benefits of the system.

4.1 METHODS OF ACTIVATING THE LACTOPEROXIDASE SYSTEM

Addition of thiocyanate/peroxide

Thiocyanate ions (in the form of sodium or potassium salt) are the substrate for lactoperoxidase and are normally added to milk at a level of approximately 14 milligrams/litre, although this could be adjusted in relation to variation in levels in milk. This is followed by addition of peroxide, either in the form of hydrogen peroxide or sodium percarbonate.

Hydrogen peroxide would be added at a level of 1-10 milligram/litre. This dose is difficult to achieve accurately and could lead to detrimental overdosing. Hydrogen peroxide is unstable and also reacts with proteins, although the latter is unlikely to cause processing problems at this concentration. Therefore sodium percarbonate (30 milligrams/litre) is recommended by Codex as the source of peroxide ions, as it leads to slower release of the active agents.

Activation kits consisting of sachets of thiocyanate and percarbonate can be obtained from a range of companies at a cost of treatment of US$0.0025–0.01 per litre of milk, and are recommended for administration by trained personnel only. It should be noted that the majority of the cost arises from packaging that limits the range of package sizes, especially for small volumes of milk. Most kits are designed for use with 50 litre batches of milk, although kits for treatment of 500 to 10,000 litres are commercially available. The major problems associated with these materials are as follows:

i) thiocyanate is hygroscopic and may deteriorate with time, although this problem may be obviated by the use of coatings or hermetically sealed containers;
ii) some sources of thiocyanate do not comply with accepted quality standards;[4]
iii) percarbonate may produce oxygen leading to 'blown' packets of activator.

Addition of glucose oxidase (1–2 milligrams/litre) to milk, following thiocyanate ions, has been demonstrated on a laboratory scale to activate the LP-s by conversion of glucose to gluconic acid and peroxide. There is usually sufficient glucose present in raw milk as a result of β-galactosidase action, particularly derived from yeasts, although addition of 2–3 grams/litre exogenous glucose is a further possibility. It is an expensive method and dose control at such low levels of addition would be very difficult.

Addition of lactic starter bacteria (catalase negative) could be used in milk for cheese-making in cases where chemical additions were unacceptable. Use of 10^4–10^5 cells/millilitre is effective, for instance in combating psychrotrophic organisms.

Addition of microorganisms (introduced deliberately or inadvertently) such as yeasts or Corynebacteria can activate the LP-s. The use of the latter following surface rinsing with thiocyanate has been shown to be effective in controlling *Listeria* on the surface of soft cheese. Autoinhibition by contaminating microorganisms may contribute to shelf-life extension in pasteurized milk and milk products where significant levels of activity remain following heat treatment (see section 4.2 next page).

Leucocytes may activate LP-s through production of hydrogen peroxide, although their presence is obviously undesirable, reflecting mastitic infection.

Hydrogen peroxide residues from disinfectant solutions following cleaning of milk containers may also activate the system.

[4] Purity criteria of thiocyanate has been specified by the Joint FAO/WHO Expert Committee on Food Additives (available from the JECFA database for food additives HYPERLINK "http://www.fao.org/ag/agn/jecfa-additives/search.html?lang=en" http://www.fao.org/ag/agn/jecfa-additives/search.html?lang=en)

4.2 THERMAL INACTIVATION OF THE LACTOPEROXIDASE SYSTEM

The kinetics of thermal inactivation of the lactoperoxidase enzyme are well established (e.g. Ramet, 2004; Barrett, Grandison and Lewis, 1999). In practical terms, batch pasteurization (e.g. 65°C/30 minutes) has little effect on enzyme activity, HTST pasteurization (72°C/15 seconds) results in retention of approximately 70% lactoperoxidase while treatment at 80°C or more (including conventional or UHT sterilisation) leads to complete destruction of the enzyme. It has been suggested (Marks, Grandison and Lewis, 2001) that this residual activity explains the fact that milk pasteurized at 72°C has a longer shelf life than milk subjected to 80°C, which has implications in cases where the milk industry may contemplate increasing severity of pasteurization conditions. In fact, it is possible that residual lactoperoxidase plays a role in the keeping quality of pasteurized milk and dairy products generally.

Lactoperoxidase activity could be used as a marker enzyme for effectiveness of HTST heat treatment because of its similarity to phosphatase in terms of thermal inactivation. The official method involves estimation of phosphatase, although this is not useful in camel milk (Ramet, Abeideirrahmane and Ould Mohammed, 2004) where phosphatase remains active following heat treatment at 82–86°C for two minutes. A lactoperoxidase assay would clearly be more appropriate as a marker in the latter case.

4.3 OTHER APPROVED METHODS OF MILK PRESERVATION

The major approved methods of milk preservation are refrigeration and/or heat treatment, although both methods have limitations with respect to processing.

REFRIGERATION

While refrigeration[5] is clearly very effective in inhibiting growth of bacteria, limited negative physical and chemical effects occur which could have small effects on processing parameters. The most important are solubilisation of β-casein, solubilisation of minerals, changes to fat crystallisation and alteration of the balance of bacteria in milk, with an increase in psychrotrophic organisms. Residual proteolytic and lipolytic enzyme activity coming from psychrotrophs following processing gives rise to problems including rancid or bitter off-flavours in products (especially cheese), gelation in UHT milk and gelation in reconstituted calf-feeding powders.

In some countries refrigeration is not feasible at some production sites because of the prohibitive cost (in terms of both initial investment and running costs), but also because of technical problems, such as the absence or unreliability of an electricity supply. The LP-s could be used as a complementary treatment where a power supply is unreliable.

[5] According to the Codex guidelines, milk for further processing should be cooled within two hours to or below 6°C when collected on a daily basis, or to or below 4 °C when not collected every day (CAC, 2004b).

Heat treatment

Obviously heating is the most effective way of destroying microorganisms and is applied to milk in treatments of varying severity (thermisation, pasteurization, sterilisation). Several negative chemical effects occur in products depending on severity of treatment. Whey protein denaturation leads to changes in functionality which can lead to problems owing to reduced syneresis of cheese curd, although high heat treatments are necessary to produce satisfactory yoghurt texture, where syneresis is undesirable. Attachment of β-lactoglobulin to β-casein on the casein micelle surface at high temperatures results in milk with reduced ability to coagulate with clotting enzymes. Hence rennet cheese-making from sterilised milk is not possible. Heating of milk leads to the Maillard reaction (between proteins and reducing sugars) giving rise to browning reactions as a result of melanoidin formation, and also to 'cooked' off-flavours. Heating of milk gives rise to insolubilisation of calcium phosphate (and complexes with proteins) which leads to fouling of processing surfaces, and may require the heated milk to be supplemented with calcium salts before cheesemaking.

It should be noted that heat treatment is more effective if the initial cell counts are minimised before processing, hence application of the LP-s prior to heating provides a complementary, possibly synergistic, combination.

4.4 EFFECTS OF THE LACTOPEROXIDASE SYSTEM ON ORGANOLEPTIC QUALITY OF MILK AND THE MANUFACTURE OF PRODUCTS

It can be surmised that use of the lactoperoxidase system might lead to limited chemical changes to the milk – e.g. through oxidation of fat and proteins. Subsequent physical effects, combined with microbiological changes could lead to negative effects on organoleptic quality of milk and milk products, and the manufacture and texture of some products. However, a report from Ponce *et al.* (2005) indicates that such effects have not been observed in practice.

It has been found that enrichment of raw milk with reagents used for lactoperoxidase activation does not modify sensory properties of the treated milk compared to control milk (Ramet, 2004). The flavour of fermented goats' milk and cheese may actually be improved as a result of the action of the lactoperoxidase changing the balance of microflora (Seifu, Buys and Donkin, 2005).

There is a clear potential for inhibition of lactic starters due to lactoperoxidase activity, resulting in reduced acid production and coagulation problems with acid-gelated products. In addition, interaction of lactoperoxidase with sulphydryl groups of proteins could alter texture of gelled products – e.g. reduction in β-lactoglobulin/β-casein interaction in yoghurt. Evidence for these phenomena is mixed. Evidence from Latin American studies suggests that the lactoperoxidase system has no negative effects on the quality of cheese and fermented products when milk has been subjected to adequate heat

treatment following the use of the LP-s (Ponce *et al*., 2005). Ozer *et al*., (2003) reported some limited effects of LP-s activation on yoghurt gel texture, while Revol-Junelles and Milliere (2005) and Seifu, Buys and Donkin (2005) reviewed the topic and found some evidence of slower rennet clotting and weaker gels in cheese, and lower acid production in yoghurt. However, the effects were generally very limited and reports are not consistent.

The sensitivity of the lactic acid starter bacteria to LP-s action mainly depends on the susceptibility of the specific strains. Susceptibility can be categorised into three groups as follows (Seifu, Buys and Donkin, 2005; Guirguis and Hickey, 1987):

- The most sensitive group of organisms which generate hydrogen peroxide, e.g. *Lactobacillus acidophilus, Lactobacillus bulgaricus;*

- Organisms that are sensitive but do not have the ability to generate hydrogen peroxide and thus require an exogenous source of hydrogen peroxide e.g. *Lactobacillus helveticus, S. thermophilus*;

- Organisms resistant to inhibition e.g. *Lactococcus lactis.*

In summary, it is concluded that any effects of LP-s activation on processing of milk are quite limited. There is no evidence that LP-s activation results in any serious negative effects.

It should be emphasised that using the LP-s to maintain the microbiological status of milk for processing should lead to superior product quality and this has been borne out by some of the FAO field trials on different fermented products (FAO, 2004a).

4.5 OTHER METHODS OF MICROBIOLOGICAL CONTROL

Microfiltration is used in some countries to reduce bacterial populations prior to pasteurization. It is feasible that it could be used as a "stand-alone" technique in the future. The process has the benefit that it is a purely physical treatment based on membrane filtration, which could circumvent many of the disadvantages of heat treatment. A disadvantage is that the diameters of fat globules and microorganisms are similar such that microfiltration is limited to skimmed milk, which can subsequently be remixed with heat treated fat-rich streams, if required. Microfiltration has also been proposed as an alternative solution to the health risks in manufacture of cheese from raw milk. However, it is unlikely that microfiltration will be adopted at present in countries where refrigeration is not routinely carried out because of technical complexities and higher costs.

High-speed centrifugation has been applied to reduce bacterial cell and spore counts in milk prior to hard and semi-hard cheesemaking. Again this is a physical process, but is unlikely to be adopted at present in developing countries because of technical complexities.

High pressure processing (400–800 MPa) has the potential to inactivate microorganisms in milk and alter the protein functionality. This has not been applied commercially.

Addition of lysozyme chlorohydrate (derived from eggs) is a permitted treatment to prevent "blowing" because of outgrowth of clostridium spores during ripening of hard and semi-hard cheeses. However, this is a limited application.

Addition of high levels of sodium chloride (3–12%) reduces water activity (A_w) of milk sufficiently to arrest bacterial growth. The technique is employed in some middle-eastern countries in the traditional manufacture of local brined cheese. Although it is a traditional process, there are many negative effects including very salty taste, micelle disruption, coagulation problems and corrosion of processing equipment. Hence the application is extremely limited.

4.6 IMPACT OF THE ADOPTION OF THE LACTOPEROXIDASE SYSTEM ON THE USE OF NON-APPROVED METHODS OF MILK PRESERVATION

A number of non-approved milk preservation methods are applied in some countries, including:

- Addition of high (300–800 milligrams/litre) levels of hydrogen peroxide, which leads to a direct bactericidal effect, but causes problems in processing because of disruption of proteins, and from a nutritional perspective it reduces the levels of vitamin A and carotenoids.
- Direct addition of antibiotics.
- Addition of ice (from water which may be contaminated), which clearly dilutes the milk.
- Transfer of chemicals from burnt wood containers to the milk.
- Alkalisation with sodium hydroxide or calcium dihydrate.
- Addition of other chemicals, including formalin or chlorine.

It is clear that lack of, or limited effectiveness of quality control procedures in developing countries leads to lack of detection of these non-approved methods. While adoption of

the LP-s has the potential to reduce the use of these non-approved methods, and hence reduce potential risk to consumer health, there is currently little available evidence to illustrate this. However, evidence from extensive studies in Cuba and Latin American countries (Ponce, 2005) suggests that use of LP-s activation has reduced the utilisation of some of the non-approved practices mentioned above.

4.7 CONCLUSIONS AND RECOMMENDATIONS

The meeting concluded based on numerous observations from laboratory and field studies that the LP-s does not induce adverse effects on the chemical, physical or sensory characteristics of raw milk and processed dairy products. Therefore, LP-s is an efficient alternative for preservation of raw milk that will be subjected to further processing. It does not preclude the need for pasteurization and does not negatively impact on, or interfere with, subsequent processing.

The LP-s can be used alone when refrigeration is not available, or in synergy with cooling or chilling and can be considered to be an efficient tool to improve the quality and quantity of milk and dairy products by maintaining the microbiological quality of raw milk.

Considering that the LP-s is technically considered as an effective method of milk preservation for further milk processing the meeting recommended that:

- The LP-s be considered as suitable to extend milk collection distances particularly in developing countries and thereby increase the amount of milk available for further processing and subsequent marketing.

- The LP-s is be used to improve the quality of processed products because of its proven bacteriostatic effect from milk collection to final processing.

5. Economic Value and Trade

In addition to the nutritional benefits of milk and its contribution to household food security, particularly in developing countries, dairying can also provide a major contribution to income generation. This is particularly important in areas where up to 80% of the total milk marketed goes through informal channels.

Refrigeration is the preferred means of milk preservation but requires high capital investment and can incur high running and maintenance costs. Use of LP-s is a reliable and economical method of preserving raw milk as compared to cooling in small-scale dairy enterprises, coupled with good hygiene and sanitation.

There is increasing regional and international trade in milk and dairy products from countries which were, in the past, net milk importers. Regional standards and equivalence are therefore of increased importance, particularly due to regional trade blocks and global trade agreements.

5.1 CURRENT SITUATION

In 2004, the total world milk output was 613 million metric tons of which 263 million metric tons was produced by developing countries – contributing about 30% share of the total world milk production, with small dairy farmers contributing about 70% of the total (NDA, 2004). The small farmer contribution to milk production may be conservative considering their share of the informal market. There was a 10.4% growth in global sales of milk and milk products recorded in 2003 (NDA, 2004). The group noted that one of the contributing factors is the rapid growth of emerging markets such as China, the Philippines and Saudi Arabia.

In 2003, FAO conducted a rapid appraisal of milk post harvest losses in five countries, including the Near East and Eastern Africa (FAO, 2004b). In Kenya, for example, the study found that a total of 15.4% of milk was lost at the farm and market level. The total national loss was estimated at 95 million litres, valued at about US$22.4 million. The losses at farm level are equivalent to US$15.4 million. Viewed against the poverty level where almost 60% of the population survive on less than US$1 a day, the loss at farm level alone is equivalent to the annual salary for 32,000 rural wage earners on US$40 per month (FAO, 2003).

Although milk production costs are low in developing countries, there can be high milk losses where ambient temperatures are high and the milk market chain lacks infrastructure and resources for refrigeration, and where there are problems with electricity

supply. The World Bank estimates that 20% of milk in developing countries is wasted. The opportunity to increase milk production and create additional income to farmers is also constrained by limited capacity for market absorption, lack of facilities to store milk (morning and evening milk) and difficulties to deliver milk on time to processing plants/collection centres.

Milk prices range from US$13 to US$50 per 100 kilograms, with a total cost of production from US$18 to US$28 per 100 kilograms of milk (IFCN, 2002). Due to low input production systems and the exchange rates, cost of milk production and milk prices are lower in developing countries. According to the FAO Dairy Outlook (FAO, 2002) the farm gate prices of milk were highest in Japan and lowest in developing countries such as Kenya, Malawi, Pakistan and Colombia (Table 3).

Table 3. Farm gate prices (cows milk) in US$/kilogram (October, 2002)

Range US$	Country (Price US$ per kilogram)
0.61 – 0.70	Japan (0.62)
0.51 – 0.60	Switzerland (0.53)
0.41 – 0.50	Mauritania (0.42)
0.31 – 0.40	Malta (0.37), Canada/Italy/Mauritius (0.35) France/Ireland/Germany (0.33), Sweden (0.31)
0.21 – 0.30	Costa Rica/Thailand/USA (0.28), Philippines/UK (0.27) Ecuador/Netherlands (0.26), Egypt (0.24), Nepal (0.22)
0.11 – 0.20	Kenya/Malawi (0.20), Pakistan/Colombia (0.18)

Source: *Calculation from FAO Dairy Outlook* (Muriuki, 2002)

Preserving milk using the most practical and economical method while maintaining its initial quality is deemed necessary to increase total milk production and marketing. This is especially relevant to developing countries through the reduction of post harvest losses of milk, promoting afternoon milking collection and the capture of more milk volume from informal markets.

5.2 THE COST OF REFRIGERATION AND THE LACTOPEROXIDASE SYSTEM

When considering the cost effectiveness of the LP-s, it should be borne in mind that it is difficult to compare with other methods applied throughout the world because costs, such as energy, vary widely and have increased significantly in recent years. It is important that such an evaluation be done on a case-by-case basis.

In the Philippines, initial investment in small-scale chilling equipment is between US$3000 and US$5000, and with the on-going cost of electricity it would not be viable to operate such equipment in a cooperative society with a 100 litre per day collection. In 1994, the total cost to cool 100 litres of milk was approximately US$0.5 compared to

US$0.35 if the LP-s is applied. LP-s preservation is cheaper and does not require a large outlay for equipment and cooling facilities (Barraquio *et al.,* 1994).

In Kenya, the cost of cooling a litre of milk ranged from US$0.017 (large scale coolers) to US$0.032 (small scale) while LP-s application was lower at US$0.014 (Wanyoike *et al.,* 2005). However, large scale milk cooling is not a solution to the problem considering the high cost of equipment, from US$197,000 to US$4 million, in addition to maintenance costs and the costs of milk collection.

In Cuba, more than 50% of the milk is not refrigerated due to, among other reasons, the high cost of cooling equipment and lack of electricity. However, the use of the LP-s has allowed significant quantities of milk, valued at US$100 million over 13 years, which would otherwise have been lost, to enter the food chain. The LP-s has proved to be effective in the dairy chain in maintaining the initial quality of the milk from the farm level through to the dairy plant. In Latin America, 30 million litres of milk was activated using the LP-s between 2000 and 2005. Fifty percent of the milk that would otherwise be lost is saved through the LP-s, amounting to a value of around US$3 million. In the Latin American region the cost of cooling a litre of milk can range from US$0.05 to US$0.1 per litre compared to a cost of US$0.0025 to US$0.05 per litre for LP-s application, again without considering the large capital outlay for investing in the cooling equipment and its maintenance.

The cost of using the LP-s compares favourably with that of cooling, particularly for smallholder dairy farmers. It has been shown that the LP-s is more cost effective than cooling in areas where milk quantities are small or there is irregular or no power supply. This is also the best way to improve the flow of milk from the farm to markets thereby creating additional income for dairy households.

5.3 INTERNATIONAL TRADE

Although milk production costs in the developing countries are lower than in developed countries, the developing countries have been net importers of milk and dairy products. However, this is slowly showing signs of change with some development of regional trade, for example among a number of the regional trade blocks in Africa including the East African Community (EAC), Common Market for Eastern and Southern Africa (COMESA), Inter-Governmental Authority on Development (IGAD) and the Southern African Development Community (SADC). Due to increased international trade in countries like Kenya in the EAC and South Africa in the SADC area, there is need for harmonisation in milk and dairy product standards to facilitate trade. Most of these countries have their national standards based on the Codex standards. It is therefore easy to harmonise their standards, although it is important that in the development of Codex standards, regional differences are taken into consideration if the standards are to continue to be of relevance to those countries.

It is difficult to estimate the loss in trading opportunities as a result of the Codex provision that the LP-s should not be used for products intended for international trade. However, the issue is not only related to trade, but also that the LP-s is not adopted in the first place because of a fear of being excluded from international markets. If products treated with the LP-s are not considered suitable for international trade then this raises doubts as to whether it is appropriate and safe to use for milk and dairy products in the domestic market. Despite this, the LP-s is applied in some countries where it is the most practical option for raw milk preservation. Kenya, for example, exports dairy products worth over US$4 million (2003 estimate) to the immediate region, and this is rising. This is the trade that could potentially be lost if they were to officially adopt the use of the LP-s and abide to the condition of not trading the milk treated with the LP-s. The meeting noted that it is likely that similar situations exist in Africa, Latin America and other developing countries.

5.4 DAIRY STANDARDS, POLICY AND THE LACTOPEROXIDASE SYSTEM

The standards developed by the Codex Alimentarius Commission are, under the WTO SPS agreement, the recognised international benchmark standards for food safety. Codex has developed a number of standards for milk and dairy products. These standards inform many of the dairy standards adopted in both developed and developing countries. National governments adopt or modify these standards depending on their national needs and dairy development policy and implementation strategies. It is important that developing world conditions are borne in mind in standard development. This would contribute to the ease with which standards are understood and can be adopted by governments and adapted under prevailing conditions within the national legal framework governing the dairy industry and milk and dairy products.

Smallholder dairy farmers play an important role in the supply of fresh milk and dairy products to growing urban centres in developing countries. To ensure the supply of the quantity of milk needed, dairy development policies need to have a choice of suitable options for milk preservation, which can be adopted by the national milk industry (Muriuki *et al.,* 2003). There are currently only two Codex approved means of preserving raw milk, i.e. refrigeration and the lactoperoxidase system of raw milk preservation. The LP-s is recognised as a cost efficient means of raw milk preservation and can be effective in reducing milk losses and expanding milk collection systems. In addition, it also appears to have significant potential for use with refrigeration as a complementary means of milk preservation. The consideration of the use of the LP-s within a national dairy development policy and strategy is therefore essential to meet the needs of producer groups, milk collectors and processors, particularly in developing and transitional countries where refrigeration is not an immediately feasible and practical option.

5.5 ECONOMIC VALUE AND IMPACT

The World Bank reported that in West Africa approximately 5 million litres of milk is

thrown away annually due to spoilage. Cuba has reported that the use of LP-s system has produced a wide range of benefits over a 13-year period. It has enabled them to get total volumes exceeding 1000 million litres of milk into the market. A conservative estimate indicated that the use of the LP-s has prevented the loss of approximately 50,000 tons of milk, which is equivalent to the annual dairy imports for the country in foreign currency. In addition it has led to the creation of employment and improvement in dairy farmers incomes (P. Ponce, personal communication, 2005).

A functional system of raw milk preservation can stimulate increased milk production to be benefit of both producers and consumers. In a country like Kenya, milk production fluctuates between seasons and, mainly only the morning milk gets into the market chain. During a high production season, there are very high milk losses due to collection logistics, exacerbated by lack of preservation systems. Evening milk is not collected due to a lack of feasible preservation systems. It has been estimated that the total amount of marketed milk would increase by about 30% through collection of evening milk. This would translate to an annual increase of over 100 million litres. An FAO study (FAO, 2005) however estimated a lower level of losses. A conservative estimate by Muriuki (H. Muriuki, personal communication, 2005) is that there would be an increase of 68 million litres of milk from market growth.

Milk markets usually pay a premium for quality milk. In Kenya, the processors pay about US$0.06 per litre for high quality milk over the going standard milk price. An increase in marketed milk, especially from the smallholder sector, would also improve livelihoods through employment, increased incomes and improved nutrition. Other issues that will need to be addressed with an increase in marketed milk include whether this will take milk away from home consumption and whether it will shift incomes from women to men. In some communities, income from milk sold within the immediate neighbourhood is controlled by women and the income from the formal sector is controlled by men.

5.6 AVAILABILITY OF THE LACTOPEROXIDASE SYSTEM COMPONENTS

Most countries with pharmaceutical facilities have the capacity to produce activators as long as they meet the specifications stipulated in the Codex guidelines and account for the purity and hygroscopic nature of percarbonate. Currently, only a few countries produce the LP-s activators, such as Sweden, Cuba and France. It would be expected that the LP-s would be more economical if the activators were made in the countries applying the system. The cost of packaging also needs to be considered given that the package alone constitutes around 40–60% of the total cost of the product.

5.7 CONCLUSIONS AND RECOMMENDATIONS

Economic benefits of dairying include household income generation that can be a major contribution to regular income and household food security and nutrition, particularly for vulnerable groups, e.g. children and women, in developing countries. Small-scale

dairy production, collection, processing and marketing are a major source of off-farm rural employment. Nevertheless, post harvest losses are a major issue in dairying in developing counties. Smallholder dairy farmers could increase their participation in worldwide milk production, processing and marketing if they could reduce their losses using any approved milk preservation method. The potential increase in the quality and shelf life of milk and dairy products may have a considerable social and economic benefit at local level. While refrigeration is the preferred means of milk preservation it does require high capital investment and can incur high running and maintenance costs for expensive equipment. Thus the use of the LP-s provides a reliable and economical alternative for preserving raw milk, particularly in small-scale dairy enterprises when coupled with good hygiene and sanitation. Its economically viablity, either as a standalone system or in combination with refrigeration, and its potential to significantly reduce milk losses and thereby increase the amount of milk collected leads to direct benefits for both milk producers and consumers.

There is increasing regional and international trade in milk and dairy products from countries which were, in the past, major milk importers. With an increasing demand and milk production growth in developing and transitional countries, regional standards are of growing importance coupled with proper hygiene and sanitation practices along the dairy chain. Such standards are often based on Codex standards as these are considered the benchmark standard under WTO for foods in international trade. However, the provision relating to the use of the LP-s makes this somewhat of an exception and is an important limitation to the adoption of the system because of the potential of being shut out of regional and international trade in these products.

Based on these conclusions the meeting recommended that:

- Small-scale dairying be promoted given its contribution to household nutrition, food security, and poverty alleviation.

- Codex Alimentarius develop milk and dairy product standards that can be easily adopted at regional or national level. Active participation of a representative range of country members should be supported in the development of standards.

- The current Codex limitation related to the use of LP-s in milk or dairy products intended for international trade be removed.

6. Overall Conclusions and Recommendations

The meeting sought to take a holistic approach to its review of the LP-s as a system of raw milk preservation taking into consideration the relevant microbiological, human health and nutrition, processing and technology and economic value and trade aspects.

The antimicrobial activity of the LP-s against a wide variety of milk spoilage and pathogenic microorganisms including bacteria, viruses, moulds, yeasts, mycoplasma and protozoa has been well documented in both laboratory and practical settings. The overall activity is primarily bacteriostatic, the extent of which is dependent on the initial total bacterial load, species and strains of contaminating bacteria and the temperature of milk. While its effectiveness against well-known milk spoilage and pathogenic microorganisms is well established, it was concluded that further studies would be useful on the efficacy of the LP-s against milk-borne viruses and emerging pathogenic microorganisms.

The efficacy of the system in raw milk from different species and under different ambient conditions was also considered. The Codex guidelines focus on the application of the LP-s to cow and buffalo milk. However, the meeting concluded that the same time-temperature combination as outlined in the Codex guidelines (CAC, 1991b) can also be applied to goat and sheep milk. The LP-s has also been shown to be effective in camel milk although the presence of other antimicrobials in this milk mean that a different pattern in terms of the level of activity at various temperatures may be observed.

An important consideration of the meeting was the impact of the LP-s on pathogenic microorganisms in milk. Based on the available evidence the meeting concluded that the LP-s does not promote the growth of pathogenic microorganisms after completion of the bacteriostatic effect[6] and there is no evidence to show that the long-term use of the LP-s would lead to any such microbiological risks, e.g. development or accumulation of toxin-producing bacteria. Furthermore, the meeting concluded that the application of the LP-s is not likely to stimulate the development of resistance to the LP-s itself or other antimicrobial agents but due to the dynamic nature of microorganisms ongoing monitoring of the situation would be reasonable.

[6] Under laboratory conditions.

The meeting gave particular consideration to data on the effectiveness of the LP-s at time-temperature combinations outside those outlined in the Codex document. It concluded that the LP-s also has a positive impact on the keeping quality of raw milk at ambient temperatures of 31– 35 °C albeit only for 4 to 7 hours. Nevertheless, this was considered important as it may mean the difference in terms of getting milk to a refrigerated collection point in a good condition particularly in areas of warm or very warm ambient temperatures. The impact of the LP-s at refrigeration temperatures was also considered, especially the ability of the system to minimise the growth of psychrotrophic bacteria. The effectiveness of the LP-s at lower temperatures led the meeting to conclude that the application of the system could be broadened to extend the period of refrigerated storage of raw milk.

The kinetics of thermal inactivation of the lactoperoxidase enzyme are well established and the time and temperature of heat treatment will determine the level of destruction of the lactoperoxidase enzyme. The meeting noted suggestions that residual lactoperoxidase activity plays a role in the keeping quality of pasteurized milk and dairy products generally. With regard to further processing of milk it was noted that while there is the potential for the LP-s to have an impact on the organoleptic quality of milk and the manufacture of products, this has not been observed in practice. Numerous observations from laboratory and field studies indicate that the LP-s does not induce adverse effects on the chemical, physical or sensory characteristics of raw milk and processed dairy products. In considering the potential impact of the LP-s on fermented products it was noted that the data on this issue was somewhat inconsistent, which appears to relate to the difference in susceptibility of the various starter culture strain to the LP-s. Where negative effects have been reported they were limited.

The meeting concluded that the LP-s has a role to play as part of an integrated system to improve milk quality and safety. It was strongly emphasised that the LP-s cannot be used to disguise poor quality milk and that the system is most effective when implemented in conjunction with good hygienic practices. While cooling and heat treatment are well recognised as effective means of milk preservation, and numerous other systems are used on a smaller scale or being developed, the expansion of milk production particularly in developing countries where appropriate infrastructure and equipment for cooling, heat treatment or other physical processes are not always possible, means that it is important that cost effective alternatives are available. The application of naturally occurring preservation systems, of which the LP-s is one, is an area that is currently being widely investigated for application in a range of different foods and at different points in the food chain. Their application is not being considered as a replacement of existing well serving technologies, such as cooling and heat treatment, but to provide complimentary alternatives, particularly at the primary production stage when the other approaches are not available, feasible or suitable.

In this context, the meeting considered that the LP-s provides a real alternative in terms of short-term raw milk preservation. The fact that it can be used without any expensive

infrastructure or equipment makes it a potentially viable option especially for many small rural milk producers. The ability to extend the shelf-life of raw milk, in a regulated way, is critical to ensuring that safe milk is made available for consumers and there is an economic benefit for the small dairy holder. Extension of the shelf-life of raw milk can ensure that it is still in a good condition when it reaches the processing facility despite long distances or poor transport infrastructure under warm or very warm ambient conditions. Milk losses are reduced again benefiting both producer and consumer.

Noting the increasing regional and international trade in milk and dairy products from countries which were, in the past, major milk importers and the increasing demand and milk production growth in developing and transitional countries, the meeting emphasised that the implementation of standards that fulfil obligations under the WTO agreements are of growing importance. Such standards are often based on Codex standards as these are considered the benchmark standard under WTO for foods in international trade. However, the provision relating to the use of the LP-s makes this somewhat of an exception and is an important limitation to the adoption of the system because of the potential of being shut out of regional and international trade in these products.

In this context the health and nutritional aspects of milk, particularly milk that had been subjected to the LP-s was considered. In terms of human health and nutrition it was firstly concluded that the advantages of the LP-s mainly result from significantly reduced spoilage losses of milk and thus improved availability of milk as a good nutrient source in the diet and benefiting both milk producers and consumers. Milk improves health and reduces morbidity and mortality from childhood disease. Therefore, the application of the LP-s could be considered as part of a system to improve public health by increasing the availability and safety of milk. The meeting reviewed the available toxicological data on the LP-s and confirmed the evaluation of the 35th JECFA that the LP-s does not present a toxicological hazard when implemented according to established Codex guidelines. The meeting also noted that very few new data have become available since the JECFA evaluation. Nevertheless, the meeting recognised the significance of iodine deficiency and emphasised that where iodine deficiency is common, public health measures to rectify this situation are needed whether or not the LP-s is used.

Overall the meeting concluded that the LP-s has numerous advantages to offer when used as part of an integrated system to improve milk quality and safety, reduce milk losses and enhance its availability. Based on the available data and an evaluation thereof, the technical meeting considered the LP-s to be a safe method of raw milk preservation. When implemented according to established Codex guidelines the meeting concluded that there is currently no scientific basis for continuing the provision related to the limitation on the international trade of LP-s treated milk and dairy products.

RECOMMENDATIONS

In making its recommendations the meeting reiterated the safety of the lactoperoxidase system of raw milk preservation when used according to the existing guidelines (CAC 13/91), recommending its use in situations when technical, economical and/or practical reasons do not allow the use of cooling facilities. Based on its deliberations the following specific recommendations were made.

To Codex

Consider expanding the guideline for the use of this system with regard to temperature of application of the LP-s to also include the temperature range from 31 °C to 35 °C for 4–7 hours and down to 4 °C for 5–6 days.

Develop milk and dairy product standards that can be easily adopted at regional or national level through the encouragement and support of active participation of a representative range of country members in the development of standards.

Remove the current provision regarding the restriction on the use of LP-s in milk or dairy products intended for international trade as the meeting found no scientific or technical basis or economic justification for the provision.

To member countries, FAO, WHO, Codex, NGOs and the dairy industry

Acknowledge the LP-s as an effective and feasible method of raw milk preservation that does not display a negative impact on the further processing of milk.

Owing to its bacteriostatic effect, give consideration to the application of the LP-s as part of a programme to improve milk hygiene and safety along the milk chain.

Consider the application of the LP-s to complement cooling in order to extend the keeping quality of raw milk and halt proliferation of milk spoilage and pathogenic microorganisms.

Use the LP-s to improve the quality of processed products based on its proven bacteriostatic effect from milk collection to final processing and in particular to extend milk collection distances in developing countries, thereby increasing the amount of milk available for marketing. This can have significant direct benefits for both milk producers and consumers.

Recognise that the use of the LP-s is an economically viable option (either standalone or in combination with refrigeration) to significantly reduce milk losses and increase milk availability.

In addition to those recommendations specific to the use of the LP-s a number of other

related issues were discussed, based on which the technical meeting made the following recommendations.

Promote the consumption of milk as a valuable source of human nutrition contributing to healthy development and growth.

Promote the contribution of small-scale dairying to household nutrition, food security, and poverty alleviation.

Implement measures to rectify iodine deficiency in recognised IDD areas accompanied by appropriate monitoring of its prevalence. Milk can also be a valuable source of iodine, providing there is adequate iodine in the diet of the milk-producing animals.

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Appendix C - Summary table comparing LP-s, refrigeration and the combination of LP-s with refrigeration

	Safety	Microbiological performance	Applicability	Cost/benefit
LP-s	No safety concern for public health when used in accordance with the Codex guidelines.	1. Primarily bacteriostatic for many milk-borne and other human pathogenic microorganisms. 2. Maintains initial milk quality for 4–7 hours (at 30 to 35°C) and up to 24–26 hours at 15°C. 3. Does not improve milk quality. 4. No long-term microbiological resistance expected.	Milk of all species. May interfere with fermentation when milk is not adequately heat treated. No significant adverse effects on the chemical, physical or sensory characteristics of raw milk and dairy products.	1. Low start-up and maintenance costs. 2. No energy requirements. 3. Can be applied in areas where refrigeration is not a viable option. 4. May increase availability of milk and dairy products. 5. Requires appropriate training of personnel for use.
Refrigeration	No safety concern for public health.	1. Primarily bacteriostatic for many milk-borne and other human patho genic microorganisms. 2. Maintains initial milk quality for several days (depending on temp. of refrigeration and initial microbial quality of milk). 3. Does not improve milk quality	Milk of all species. Limited negative physical and chemical effects.	1. Extends keeping time of milk by several days. 2. Nothing added to milk. 3. Requires electricity. 4. Relative high cost for initial investment and maintenance.
Refrigeration with the LP-s	No safety concern for public health when used in accordance with the Codex guidelines.	1. Primarily bacteriostatic for many milk-borne and other human pathogenic microorganisms. 2. Maintains initial milk quality for 5–6 days at 4°C. 3. Does not improve milk quality. 4. No long-term micro biological resistance expected.	Milk of all species.	1. Increases shelf-life of milk and dairy products as compared to refrigeration alone 2. Minimal increase in cost.

Appendix D - Thiocyanate exposure based on the GEMS/Food regional diets both with and without lactoperoxidase treated milk

THIOCYANATE EXPOSURE WITHOUT LP-S USING FOOD SUPPLY OF GEMS/FOOD REGIONAL DIETS IN MILLIGRAMS/YEAR

GEMS/Food Consumption Cluster Diets[7]	A	B	C	D	E	F	G	H	I	J	K	L	M
Brassica vegetables	87.8	1001.5	379.1	1761.9	1267.1	1125.5	1071.2	203.5	492.0	80.4	173.3	2240.2	814.4
Tomato	19.6	370.0	236.0	121.4	63.2	74.4	47.1	63.1	29.9	25.0	71.2	19.9	180.5
Cassava	971.2	0.0	0.1	0.0	0.0	0.0	62.4	96.5	685.3	1128.7	230.9	79.2	2.6
Lima beans, dry	0.0	4.8	4.7	17.4	0.0	0.0	9.5	32.9	0.0	0.0	0.0	15.4	2.9
Milk only	344.2	953.3	396.6	1512.6	898.0	1189.4	330.1	604.0	408.1	511.6	1038.4	285.5	1439.7
Total thiocyanate exposure	1422.9	2329.7	1016.5	3413.4	2228.3	2389.3	1520.3	999.9	1615.3	1745.7	1513.9	2640.2	2440.1
Total thiocyanate exposure (milligrams/day)	**4.0**	**6.5**	**2.8**	**9.5**	**6.2**	**6.6**	**4.2**	**2.8**	**4.5**	**4.8**	**4.2**	**7.3**	**6.8**

THIOCYANATE EXPOSURE ADDING LP-S USING FOOD SUPPLY OF GEMS/FOOD REGIONAL DIETS IN MILLIGRAMS/YEAR[8]

Brassica vegetables	87.8	1001.5	379.1	1761.9	1267.1	1125.5	1071.2	203.5	492.0	80.4	173.3	2240.2	814.4
Tomato	19.6	370.0	236.0	121.4	63.2	74.4	47.1	63.1	29.9	25.0	71.2	19.9	180.5
Cassava	971.2	0.0	0.1	0.0	0.0	0.0	62.4	96.5	685.3	1128.7	230.9	79.2	2.6
Lima beans, dry	0.0	4.8	4.7	17.4	0.0	0.0	9.5	32.9	0.0	0.0	0.0	15.4	2.9
Milk only	1307.9	3622.5	1507.2	5747.9	3412.3	4519.6	1254.3	2295.0	1550.7	1944.0	3945.8	1084.9	5470.7
Total thiocyanate exposure*	2386.6	4998.9	2127.1	7648.7	4742.6	5719.5	2444.5	2691.0	2757.9	3178.2	4421.4	3439.5	6471.2
*incl. 100% LP-s treated milk (milligrams/year)													
Total thiocyanate exposure (milligrams/day)	**6.6**	**13.9**	**5.9**	**21.2**	**13.2**	**15.9**	**6.8**	**7.5**	**7.7**	**8.8**	**12.3**	**9.6**	**18.0**

[7]For complete list of country assignment codes (listed A-M above) see http://www.who.int/foodsafety/chem/gems/en/index1.html

[8]Mean exposure of sodium thiocyanate has been estimated by multiplying the mean consumption of the 13 GEMS/Food regional diets with the mean concentration in selected foods.

Appendix E - Food supply according to GEMS/Food regional[9] diets in kilograms/year

CODE	GEMS	NOTES	A	B	C	D	E	F	G	H	I	J	K	L	M	Sodium thiocyanate or HCN in milligram/ kilogram
VB 40	Brassica vegetables[10]	(14)	2.2	25.0	9.5	44.0	31.7	28.1	26.8	5.1	12.3	2.0	4.3	56.0	20.4	40[11]
VO 448	Tomato[12]	(9)	9.8	185.0	118.0	60.7	31.6	37.2	23.5	31.6	15.0	12.5	35.6	9.9	90.3	2
VR 463	Cassava[13]	(1)	242.8	0.0	0.0	0.0	0.0	0.0	15.6	24.1	171.3	282.2	57.7	19.8	0.7	4[14]
VD 534	Lima bean (dry)[15]		0.0	0.2	0.2	0.7	0.0	0.0	0.4	1.3	0.0	0.0	0.0	0.6	0.1	25[16]
ML 106	Milk[17]	(1)(2)	68.8	190.7	79.3	302.5	179.6	237.9	66.0	120.8	81.6	102.3	207.7	57.1	287.9	5 (19 with LP-s)
A0 31	**Total Milk & Milk Products**		**70.5**	**223.4**	**87.9**	**317.4**	**249.7**	**301.4**	**66.6**	**136.2**	**85.6**	**103.5**	**211.7**	**63.9**	**6.8**	

[9]For complete list of country assignment codes (listed A-M above) see http://www.who.int/foodsafety/chem/gems/en/index1.html

[10]Food Standards Australia and New Zealand, 2002

[11]Cooked (60% leaking into cooking water).

[12]Tonacchera, *et a* ., 2004

[13]WHO, 1993

[14]Cooked (1% of raw)

[15]WHO, 1993

[16]Cooked (1% of raw)

[17]Introduction 1.1 of document CAC/GL 13-1991 (CAC, 1991b)

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Lactoperoxidase is an enzyme that is naturally present in milk. The activation of the lactoperoxidase in the presence of hydrogen peroxide and thiocyanate, both of which are naturally present in milk in varying concentrations has a bacteriostatic effect on raw milk and effectively extends the shelf life of raw milk for 7–8 hours under ambient temperatures of around 30°C or longer at lower temperatures. Such an extension in shelf life particularly under warm ambient conditions can allow adequate time for the milk to be transported from the collection point to a processing centre without refrigeration.

Codex adopted the "Guidelines for the preservation of raw milk by use of the lactoperoxidase system in 1991 to facilitate the application of its use in situations when technical, economical and/or practical reasons do not allow the use of cooling facilities.

However, at that time the Codex Alimentarius Commission also agreed to emphasise that the system should not be used for products intended for international trade. In the intervening years concerns have been raised by numerous countries as to why the system can be applied for products in domestic but not international trade.

In order for Codex to reconsider this issue they requested FAO and WHO to convene a technical meeting to review the most recent scienticic information on the risks and benefits of the lactoperoxidase system.

This report provides the output of that meeting including a summary of the most recent information relating to the use of the lactoperoxidase system for raw milk preservation and the discussions and recommendations of the technical meeting.

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Annex 5

Pages 000128-000230 have been removed in accordance with copyright laws. The removed reference citations are:

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