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Original Submission

GRAS Claim Notification

Primex Chitosan

Submitted by:

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January 31, 2001

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Letter

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January 31, 2001

Dr. Laura Tarantino
Acting Director
Office of Premarket Approval
Center for Food, Safety and Nutrition HFS 200
Food and Drug Administration
200 C St. SW
Washington, DC 20204

Dear Dr. Tarantino:

In accordance with the proposed rule for Substances Generally Recognized as Safe, which was published in the *Federal Register* at Vol. 62, No. 74 on April 17, 1997, Primex Ingredients ASA (Primex) of Avaldsnes, Norway submits a notice of a claim for exemption for the general use in foods of chitosan. Chitosan is a natural polysaccharide, composed mainly of glucosamine, 2-amino-2-deoxy-β-D-glucose. It occurs widely in nature in the cell walls of fungi, and as a breakdown product of chitin.

Commercially, Primex chitosan is prepared by deacetylating chitin, extracted from the shells of the arctic shrimp, *Pandalus borealis.* The fact that chitosan is a natural substance, and can be derived from a natural substance, allows it to be considered a candidate for GRAS status. The structure of chitin is similar to that of cellulose, and chitosan has long been considered a source of dietary fiber, as it cannot be completely broken down in the human digestive tract. On a molecular basis, chitosan contains an amino group, as well as primary and secondary hydroxyl groups. These three functional groups form the basis for its functionality in foods.

A GRAS Report in support of the safe use of shrimp-derived chitosan in foods was prepared by Primex, and was reviewed on June 8, 2000 by a panel of experts qualified by training and experience. Those experts concurred with Primex's determination that shrimp-derived chitosan is safe for general use in foods.

On December 19, 2000 Primex personnel presented the salient points of the GRAS Report to officials from the Center for Food Safety and Applied Nutrition

(CFSAN), Office of Premarket Approval. At that time, the format for the attached notice of a claim for premarket exemption based on a GRAS determination under proposed §170.36 was discussed.

Primex has prepared a Notification document in triplicate, which accompanies this letter. Primex would appreciate notice of the receipt of this document, and looks forward to the agency's concurrence that there is a sufficient basis for the GRAS determination of shrimp-derived chitosan.

Thank you very much for your attention to this matter. If you have any questions regarding the content of the Notification, you may reach me at the number listed above.

Sincerely,

Lee B. Dexter
Technical Consultant

CC: Dr. Linda Kahl, CFSAN
Dr. Robert Merker, CFSAN
Mr. Steve Dillingham, Norwegian Trade Council
Mr. Bjarte Langhelle, Primex
Mr. Thore Ivar Thorsen, Primex
Mr. Sigurd Mølstre, Primex

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Primex Ingredients ASA
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Claim

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Section I GRAS Notification Claim

Primex Ingredients ASA
GRAS Notification

Introduction

Chitosan is a polysaccharide, composed mainly of glucosamine, 2-amino-2-deoxy-β-D-glucose. Chitosan occurs widely in nature in the cell walls of fungi, and as a breakdown product of chitin. Commercially, chitosan is prepared by deacetylating chitin, which is the second most abundant natural polymer on earth [Shahidi, *et al.,* 1999 Vol 8 Tab 145]. The fact that chitosan is a natural substance, and is derived from a natural substance, allows it to be a candidate for GRAS status as a food ingredient. The structure of chitin is similar to that of cellulose, and chitosan has long been considered a source of dietary fiber, as it cannot be completely broken down in the human digestive tract [Omrod, 1998 Vol 7 Tab 112]. Chitosan has three functional groups, an amino group, as well as both primary and secondary hydroxyl groups, which are the basis for its functionality in foods [Shahidi, *et al.,* 1999 Vol 8 Tab 145].

Primex Ingredients ASA (Primex) of Avaldsnes, Norway has developed a unique process to manufacture chitosan from the arctic shrimp, *Pandalus borealis.* A consistent source of raw material and well-designed manufacturing practices have made it possible to use chitosan more widely as a food ingredient. This Notification document contains the information required in proposed 170.36 to allow the FDA to evaluate whether the submitted notice provides a sufficient basis for a generally recognized as safe (GRAS) determination. This document is being submitted by Primex Ingredients ASA of Avaldsnes, Norway.

In compliance with 21 CFR § 170.30, Primex determined that Primex chitosan can be considered GRAS when used in accordance with current Good Manufacturing Practices. Primex wishes to voluntarily notify the Center for Food Safety and Applied Nutrition (CFSAN) of that determination, and according to proposed § 170.36, the company is submitting the following GRAS exemption claim.

Primex Ingredients ASA has prepared a twelve-volume GRAS Report, which forms the basis for the information found in this notification. The company also commissioned a panel of experts (Expert Panel), qualified by scientific training and experience to assess the safety of food ingredients, who critically evaluated the chitosan GRAS Report as well as other data and information relevant to the use and safety of this ingredient. In a meeting held on June 8, 2000, the panel concurred with the company's determination that Primex chitosan can be considered generally recognized as safe for general use in food. Based on the data and information contained in the Report and the

Section I GRAS Notification Claim

opinion of the expert panel (which is attached to this notification), Primex explicitly accepts responsibility for the GRAS determination of Primex chitosan, known commercially as ChitoClear™.

Section I
GRAS Exemption Claim

Primex Ingredients ASA hereby notifies the U.S. Food and Drug Administration that the use of Primex chitosan (as a food ingredient is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act, because Primex has determined that such use is GRAS.

A. Notifier:

Primex Ingredients ASA
% Norwegian Trade Council
800 Third Avenue, 23rd Floor
New York, New York, USA 10022
Tel: (212) 421-9210
Fax: (212) 838-0374

B. Common or Usual Name:

Shrimp-derived Chitosan
Shrimp Chitosan

C. Applicable Conditions of Use:

Applications for chitosan include general use in foods as a multiple-use direct additive. The ingredient should be used under the conditions of current Good Manufacturing Practice.
In order to classify the various effects ingredients may have in food, FDA has published a list of 32 physical or technical functional effects for which direct food ingredients may be added to food. These are codified at 21 CFR §170.3 (o) (1-32). Applications for chitosan are covered under several of the following terms as listed under 21 CFR §170.3 (o).

(3) "Antioxidants": Substances used to preserve food by retarding deterioration, rancidity, or discoloration due to oxidation.
(5) "Curing and pickling agents": Substances imparting a unique flavor and/or color to a food, usually producing an increase in shelf life stability.
(6) "Dough strengtheners": Substances used to modify starch and gluten, thereby producing a more stable dough, including the applicable effects listed by the National Academy of Sciences/National Research Council under "dough conditioner."

(8) "Emulsifiers and emulsifier salts": Substances which modify surface tension in the component phase of an emulsion to establish a uniform dispersion or emulsion.
(14) "Formulation aides": Substances used to promote or produce a desired physical state or texture in food, including carriers, binders, fillers, plasticizers, film-formers, and tableting aids, etc.
(16) "Humectants": Hygroscopic substances included in food to promote retention of moisture, including moisture-retention agents and antidusting agents.
(20) "Nutrient supplements": Substances which are necessary for the body's nutritional and metabolic processes.
(24) "Processing aids": Substances used as manufacturing aids to enhance the appeal or utility of a food or food component, including clarifying agents, clouding agents, catalysts, flocculents, filter aids, and crystallization inhibitors, etc.
(28) "Stabilizers and thickeners": Substances used to produce viscous solutions or dispersions, to impart body, improve consistency, or stabilize emulsions, including suspending and
bodying agents, setting agents, jellying agents, and bulking agents, etc.
(29) "Surface-active agents": substances used to modify surface properties of liquid food components for a variety of effects, other than emulsifiers, but including solubilizing agents, dispersants, detergents, wetting agents, rehydration enhancers, whipping agents, foaming agents, and defoaming agents, etc.
(31) "Synergists": Substances used to act or react with another food ingredient to produce a total effect different or greater than the sum of the effects produced by the individual ingredients.
(32)"Texturizers": Substances which affect the appearance or feel of the food.

D. Basis of the GRAS Determination

The basis of the GRAS determination for Primex Ingredients chitosan was the use of scientific procedures.

E. Availability of Data and Information and Key to References

The data and information that are the basis of the GRAS determination for Primex Ingredients chitosan will be available at the address of the notifier listed above. However, throughout this Notification, citations to the published literature, which were included in the 12-volume GRAS Report are denoted as follows: [Author (*et al*), Year, Tab (number) Volume (number)]. In order to facilitate review of this document a

Section I GRAS Notification Claim

complete list of references from the chitosan GRAS Report is included in Appendix 2 as a key. Recently identified references, which were not included in the GRAS Report are shown between parentheses ( ) within the text of this document and are given in a standard bibliographic form.

F. Signature of an official for Primex Ingredients ASA

Official for Primex Ingredients ASA Date

January 31, 2001

Lee B. Dexter, Technical Consultant

Notification Section II

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Section II
Chemical Identity

A. Common or Usual Name and Identity

Shrimp-derived Chitosan
Shrimp Chitosan

B. Formal Names (IUPAC or Chemical Abstracts Names)

Chitosan

1. Polyglucosamine
2. Poly-[1→ 4] – ß-D-glucosamine
3. Poly 2-Amino-2-deoxy—D-Glucopyranose
4. Binary heteropolymer of ß (1→ 4)-2-Amino-2-deoxy—D-Glucopyranose and ß (1→ 4)-2-deoxy-2-acetamido-D-glucopyranose

Molecular Subunits

1. D-Glucosamine = 2-Amino-2-deoxy-D-Glucopyranose (GlcN)
2. N-Acetyl-D-glucosamine = 2-deoxy-2-acetamido-D-glucopyranose (GlcNAc)

C. Synonyms, Other Common Names, Tradenames

1. Trademark: ChitoClear™ is a pending trademark name associated with Primex Ingredients ASA chitosan.

2. Primex Chitosan

3. Deacetylated Chitin

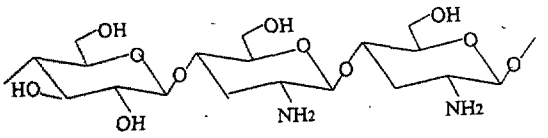
D. Chemical Formulae, Structures and Molecular Weights

1. Empirical Formula $(GlcN)_n + (GNAc)_m$
$(C_6H_{11}\ N_1O_4)_n + (C_8H_{13}\ N_1O_5)_m$

Chitosan is a polysaccharide composed of GlcN and GlcNAc linked with ß (1→ 4)-glycosidic linkages.

Section II Chemical Identity of Chitosan

2. Structural Formula



3. Molecular Weight

Most abundant species: ~1 million Daltons
[Wu, *et al.,* 1976, Vol 11 Tab 220]

E. Chemical Abstracts Service Registry Number (CAS Registry No.)

CAS Number 9012-76-4

F. Quantitative Composition of Primex Chitosan

1. Product Identity

The Primex chitosan product, ChitoClear™, is a binary heteropolymer of ß (1→ 4)-2-Amino-2-deoxy—D-Glucopyranose and ß (1→ 4)-2-deoxy-2-acetamido-D-glucopyranose produced by the deacetylation of chitin. Chitosan is obtained by the process of removing a sufficient number of acetyl groups (CH_3-CO) from chitin, so that the molecule becomes soluble in most diluted acids. This process, called deacetylation, releases amine groups (NH) and results in chitosan, a heteropolymer with cationic characteristics. Chitosans are typically characterized by two principal factors: viscosity and degree of deacetylation. Various investigators have suggested that chitosan and chitin be defined as soluble or insoluble in 0.1 M acetic acid, or by degree of deacetylation, where a substance with >20 % of deacetylation would be defined as chitosan [Shahidi *et al.,* 1998 Vol 8 Tab 144 and Shahidi, *et al.,* 1999 Vol 8 Tab 145].

The Primex product is composed of ≥ 90% dry matter, less than 1.0 % ash, and negligible amounts of protein and fat. The percent deacetylation of the chitosan (as compared to chitin) is greater than 70%, and the turbidity rating is ≤50 NTUs. The product is further characterized by a solubility of ≥98% in 1.0 % acetic acid. Lead levels are <0.1ppm, and mercury levels are below the level of detection of 5 ppb. Microbial limits have been set to meet food grade specifications (Tables 1-3). The product occurs in several food grade forms

characterized by their degree of deacetylation (>70 – >95%), and polymerization. ChitoClear™ FG is a food grade product defined as having > 70% deacetylation. ChitoClear™ FG95 has undergone extended deacetylation to yield a product with a degree of deacetylation >95%, and ChitoClear™ FGLV is a low viscosity chitosan.

Table 1
Final Product Specifications for Primex FG Chitosan
Product: Off white chitosan made from fresh shrimp shells, *Pandalus borealis.*

Food Grade	
Chemical Parameters	
Dry Matter	>90 %
Moisture	<10 %
Ash	<1.0%
Degree of Deacetylation	>70%
Solubility	>99% (in 1% acetic Acid)
Turbidity of the Solution	< 50 NTUs
Viscosity	≥5 (mPas (cPs)) 1% solutions in 1% acetic acid measured on a Brookfield LVT viscometer, 25°C, appropriate spindle at 30 rpm
Lead	<0.1 ppm
Mercury	<5 ppb
Flakes	0.1 – 6 mm
Micromilled Powder	Min. 100% < 60 mesh,
Microbiological Parameters	
Total Plate Count	≤ 1000 CFU/g
Eschericia coli	Negative
coliform Organisms	Negative
Salmonella sp. 25 grams	Negative
Yeast and Mold	≤ 100 CFU/g

Section II Chemical Identity of Chitosan

Table 2
Final Product Specifications for Primex FG95 Chitosan
Product: Off white chitosan made from fresh shrimp shells, *Pandalus borealis.*

Food Grade	
Chemical Parameters	
Dry Matter	>90 %
Moisture	<10 %
Ash	<1.0%
Degree of Deacetylation	>95%
Solubility	>99% (in 1% acetic Acid)
Turbidity of the Solution	< 50 NTUs
Viscosity	≥5 (mPas (cPs)) 1% solutions in 1% acetic acid measured on a Brookfield LVT viscometer, 25°C, appropriate spindle at 30 rpm
Lead	<0.1 ppm
Mercury	<5 ppb
Flakes	0.1 – 6 mm
Micromilled Powder	Min. 100% < 60 mesh
Microbiological Parameters	
Total Plate Count	≤ 1000 CFU/g
Eschericia coli	Negative
coliform Organisms	Negative
Salmonella sp. 25 grams	Negative
Yeast and Mold	≤ 100 CFU/g

Section II Chemical Identity of Chitosan

Table 3
Final Product Specifications for Primex FGLV Chitosan
Product: Off white chitosan made from fresh shrimp shells, *Pandalus borealis.*

Food Grade	
Chemical Parameters	
Dry Matter	>90 %
Moisture	<10 %
Ash	<1.0%
Degree of Deacetylation	>70%
Solubility	>99% (in 1% acetic Acid)
Turbidity of the Solution	< 50 NTUs
Viscosity	5-30 (mPas (cPs)) 1% solutions in 1% acetic acid measured on a Brookfield LVT viscometer, 25°C, appropriate spindle at 30 rpm
Lead	<0.1 ppm
Mercury	<5 ppb
Flakes	0.1 – 6 mm
Micromilled Powder	Min. 100% < 60 mesh,
Microbiological Parameters	
Total Plate Count	< 1000 CFU/g
Eschericia coli	Negative
coliform Organisms	Negative
Salmonella sp. 25 grams	Negative
Yeast and Mold	< 100 CFU/g

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2. Analysis of Five Lots of Primex Ingredients Chitosan

Five (5) lots of Primex Ingredients' chitosan, ChitoClear™ FG95, manufactured from over a period of time, showed that product dry matter, as determined by a standardized drying method was greater than 95.0%. Ash content ranged from 0.1% to 0.6% [Table 4 below]. The degree of deacetylation was greater than 95.6%. The solubility in a 1.0% acetic acid solution was greater than 99.0%. Turbidity was less than 18 NTUs, and the viscosity was greater than 5 cps. Microbiological analyses showed that the product contained less than 1000 CFUs per gram, was negative for *E. coli,* coliform organisms, and *Salmonella sp.*(based on 25 grams), and yeast and mold counts were less than 100 per gram. Heavy metals, mercury and lead were measured on separate lots (Table 6) [Primex Ingredients ASA 2000, Vol 7 Tab 122]. Concentrations were calculated on a dry weight basis. Zinc, measured on 4 lots was present at < 1 μg/g. Arsenic measured on 8 lots totaled <0.1 μg/g. Cadmium, also measured on 8 lots was present in concentrations of< 0.005 μg/g, and lead on the basis of 10 lots was present at < 0.095 μg/g.

Section II Chemical Identity of Chitosan

Table 4
Product: Chitosan Made from Shrimp Shells, *Pandalus borealus.*
Mean Analyses of 5 Lots[1]

Batch No.	Dry Matter	Ash	Degree of Deacetylation	Solubility[2]	Turbidity	Viscosity[3]
TM 807	96.8%	0.3%	97.5 % (Titration-Method)	>99.9% (in 1% acetic acid	13 NTUs	45 (mPa.S(cPs))
TM 808	96.8%	0.1%	95.6% (Titration-Method)	>99.9% (in 1% acetic acid	17 NTUs	68 (mPa.S(cPs))
TM 809	96.6%	0.2%	98.0% (Titration-Method)	>99.9% (in 1% acetic acid	14 NTUs	44 (mPa.S(cPs))
TM 813	95.2%	0.3%	96.4% (Titration-Method)	>99.9% (in 1% acetic acid	18 NTUs	66 (mPa.S(cPs))
TM 822	97.9%	0.6%	96.6% (Titration-Method)	>99.9% (in 1% acetic acid	15 NTUs	53 (mPa.S(cPs))
Mean Of Means	96.66%	0.3%	96.82%	>99.9%	15.4 NTUs	55.2 cPs

Form: Micromilled Powder: 100% less than 100 mesh, 50% less than 150 mesh

[1] Raw data is shown in Volume 7 Tab 121.
[2] 1% solutions in 1% acetic acid
[3] Measured on a Brookfield LVT viscometer, 25C, appropriate spindle at 30 rpm.

Section II Chemical Identity of Chitosan

Table 5
Microbiological Parameters

Batch No.	Total Plate Count	*Escherichia coli*	Coliform Bacteria	*Salmonella* / 25 Grams	Mold and Yeast
TM 807	<1000/g	Negative	Negative	Negative	<100/g
TM 808	<1000/g	Negative	Negative	Negative	<100/g
TM 809	<1000/g	Negative	Negative	Negative	<100/g
TM 813	<1000/g	Negative	Negative	Negative	<100/g
TM 822	<1000/g	Negative	Negative	Negative	<100/g
Mean of Means	<1000/g	Negative	Negative	Negative	<100/g

Table 6
Primex Chitosan Metals Analysis

Element	No. of Lots	Mean Value (µg/g) dry wt.
Zinc	4	<1
Arsenic	8	<0.1
Cadmium	8	<0.005
Lead	10	0.095
Mercury	12	<0.005

G. Manufacturing Process

1. Introduction

In general chitosan is prepared by deacetylation of chitin, which, other than cellulose, is the most abundant carbohydrate biopolymer on earth. The most common sources of chitin are crustacean shells, especially shrimp, crabs, and Antarctic krill. The reproducibility of the deacetylation process is affected not only by chemical reaction parameters but also by the biological origin of the raw material [Shahidi, *et al.*, 1999 Vol 8 Tab 145]. The properties of the nitrogen-containing polycationic polysaccharide depend largely on the degree of acetylation (DA), molecular mass, and chain conformation of the polymer. Knowledge of the degree of acetylation is an essential parameter in the characterization of chitosan.

Hwang, *et al.*, 1995, stated that the molecular weight and DA are greatly affected by the concentration, time and temperature of the deacetylation reaction [Hwang, *et al.*, 1995, Vol 5 Tab 58, and Wu, *et al.*, 1976, Vol 11 Tab 220]. Preparation of chitin from crab shell as practiced by Hwang, *et al.*, 1995 consists of demineralization with a 5% HCl treatment followed by deproteinization with 5% NaOH. Ash content and nitrogen content are measured to determine the quality of chitin after processing. A hot 50% NaOH solution is used for the deacetylation process of chitin, and the DA, molecular weight, and viscosity of the chitosan were measured by the researchers. The concentrated NaOH solution can be reused in some manufacturing schemes. Hwang, *et al.*, 1995, found that the degree of demineralization was not significantly different at 50°C and 70°C after 30 minutes of reaction time. Deproteinization decreased as process time increased. The nitrogen content reached 6.92% after 90 minutes at 80°C, which was similar to the theoretical nitrogen content of chitin. After 2 hours of reaction time, the degree of deacetylation reached by this process was 82.84% and the level continued to increase as the reaction time increased. Viscosity and molecular weight increased as the number of recycles of concentrated NaOH increased. The authors concluded that the chemical, biological, and physical properties of chitosan depend on the degree of deacetylation and the molecular size of the molecule [Hwang, *et al.*, 1995, Vol 5 Tab 58, and Wu, *et al.*, 1976, Vol 11 Tab 220].

The physical properties of chitosan relate to its polyelectrolyte and polymeric character. It is insoluble in water, alkaline solutions at pH levels above about 6.5, and organic solvents. However, chitosan

dissolves readily in dilute solutions of many organic acids, including acetic, formic, tartaric, and citric acids [National Toxicological Survey, 2000, Vol 7 Tab 108].

Data submitted to the United States Environmental Protection Agency for 1994 indicated that chitosan production was less than 10,000 pounds per year [National Toxicological Survey, 2000 Vol 7 Tab 108]. A world consumption estimate for the same year stated that there were more than 1,000 tons of chitin and chitosan being produced, with 800 tons of that amount being used in Japan.

2. Production Process of Primex Chitosan

Primex has developed a novel production process for chitosan based on chitin from North Atlantic shrimp shells (*Pandalus borealis*). The company has secured a supply of this raw material through long-term partnership agreements with the Norwegian shrimp fishing and peeling industry.

Technological developments of new production equipment has allowed the developing Norwegian shrimp peeling industry to process in full accordance with HACCP standards, ensuring food grade shell raw material.

Prior to Primex' collection of the shrimp shells from nearby shrimp peeling plants, the shrimp is frozen (immediately upon harvest) at a temperature of less than -10°C onboard the shrimp fishing vessels. After the ships return to port, the shrimp is kept frozen (< -10°C) at the shrimp peeling plants. Thawing occurs just prior to an automated machine peeling process.

Primex's production processes are optimized with the aid of specialized production equipment. All quality control procedures are based on a single source of shrimp shell raw material. All process equipment is designed by Primex personnel, and the entire process is fully automated from the receipt of the shrimp shells at Primex production plant to the final product. The product obtained from this process is currently being sold in Japan and Korea as a food ingredient or additive, and in other countries for non-food uses. It should be noted that all production raw materials are food grade quality and there are a variety of critical control points used to ensure the wholesomeness of the product.

Section II Chemical Identity of Chitosan

The following is a description of the production process for Primex chitosan.

a) Collection of Raw Material

The shells are collected and transported to Primex processing plants in specially designed 10-ton insulated containers. During transportation the shells are kept refrigerated (max. 7°C upon receipt at Primex plant), and processing of the shells begins within no more than 24 hours after peeling. Upon receipt, Primex quality control personnel perform a visual inspection of the raw material to check the quality of the shells. The operators evaluate whether the shells have started to decompose and whether they have begun to generate an odor. If the shells do not meet the inspection criteria, they are rejected and dumped at a local waste site [Primex Ingredients ASA Production Process Vol 8 Tab 121]. Suitable begin processing after inspection.

b) Deproteinization

The shrimp shells are stirred in specialized stainless steel tanks for a defined time in a solution of NaOH and water calculated to remove the protein from the shells. The water/NaOH solution is removed by passing the mixture through a mechanical filter.

c) Washing I

Fat and color are rinsed from the shrimp shell chitin with EU-classified tap water in specialized stainless steel tanks through sequential washing steps, which involve heating the shells and water to an appropriate temperature and adjusting the pH to a specific value.

d) Demineralization

The shrimp shell chitin is stirred in specially constructed stainless steel tanks in a solution of HCL and water at an appropriate pH and temperature. Process operators visually monitor the reaction. Laboratory analysis of ash content should not exceed 2.0 %.

e) Washing II

The demineralized chitin is washed with heated and pH adjusted tap water. The purpose of this rinse is to remove soluble fat and color. The pH value after washing should be >6.

f) Deacetylation

The chitin is added to a solution of NaOH of appropriately high concentration in order to remove the acetyl groups from the chitin. The chitin/NaOH solution is stirred at an appropriate temperature for an appropriate time in special stainless steel tanks. Analysis of the degree of deacetylation is conducted in the Primex laboratory. After deacetylation is completed the product is filtered through a mechanic filtering system.

g) Washing III

After the process of deacetylation, the mixture is rinsed with tap water in sequential steps to remove the NaOH and to reduce the pH value to less than 8. After washing is completed, the product is filtered through a mechanic filtering system.

h) Drying and Milling

The product can either be 1) dried directly into flake form appearance or 2) dried and milled in one step into powder appearance.

1. The chitosan wet flakes are dried in a Turbo dryer, until the desired moisture has been achieved. The moisture content of the chitosan is reduced to less than 10% before drying is completed.

2. The chitosan wet flakes are dried and milled in a one step process using a designer made Turbo drier/milling system, until the desired moisture and particle size is achieved. The moisture content of the chitosan is reduced to less than 10% before drying is completed.

i) Sieving and Packaging

After sieving through an appropriate wire filter screen, the product is collected in a hopper, and pumped into batch tanks. The production batches are then packed in large double lined bags, retainer samples are collected, and the product batches are stored until all the analyses are complete. Upon completion of the analyses, the product is packed in standardized cardboard cartons with inner polyethylene bags and labeled. When packaging is completed, the product is stored in accordance with food grade conditions at the Primex warehouse.

Extended deacetylation

For the production of ChitoClear FG95, which is a highly deacetylated form of chitosan, additional deacetylation processing is required. After the deacetylation processing step described in section f) above, the chitosan is pumped into a separate processing tank, and "fresh" NaOH of a known concentration is added. The mixture of chitosan / NaOH is stirred at a known rpm, at an appropriate temperature and for an appropriate time. Analysis of the degree of deacetylation is conducted in the Primex laboratory. After deacetylation is complete, the product is filtered through a mechanical filtering system.

Depolymerization

For the production of ChitoClear FGLV, which is a low viscosity form of chitosan, an additional depolymerization step is required. The depolymerization step is performed after the third washing step described in step g). Dilute H_2O_2 at specific temperatures is used in sequential steps in the same procedure as described for the washing steps. After depolymerization, additional washing steps are performed to neutralize the pH.

The sequence of production steps specific for each of the Primex chitosan products is shown below in Table 7 and Figure 1 below. Specific market categories for the various products are shown below in Table 8.

Table 7
Specific Production Steps for the Primex Chitosan Products

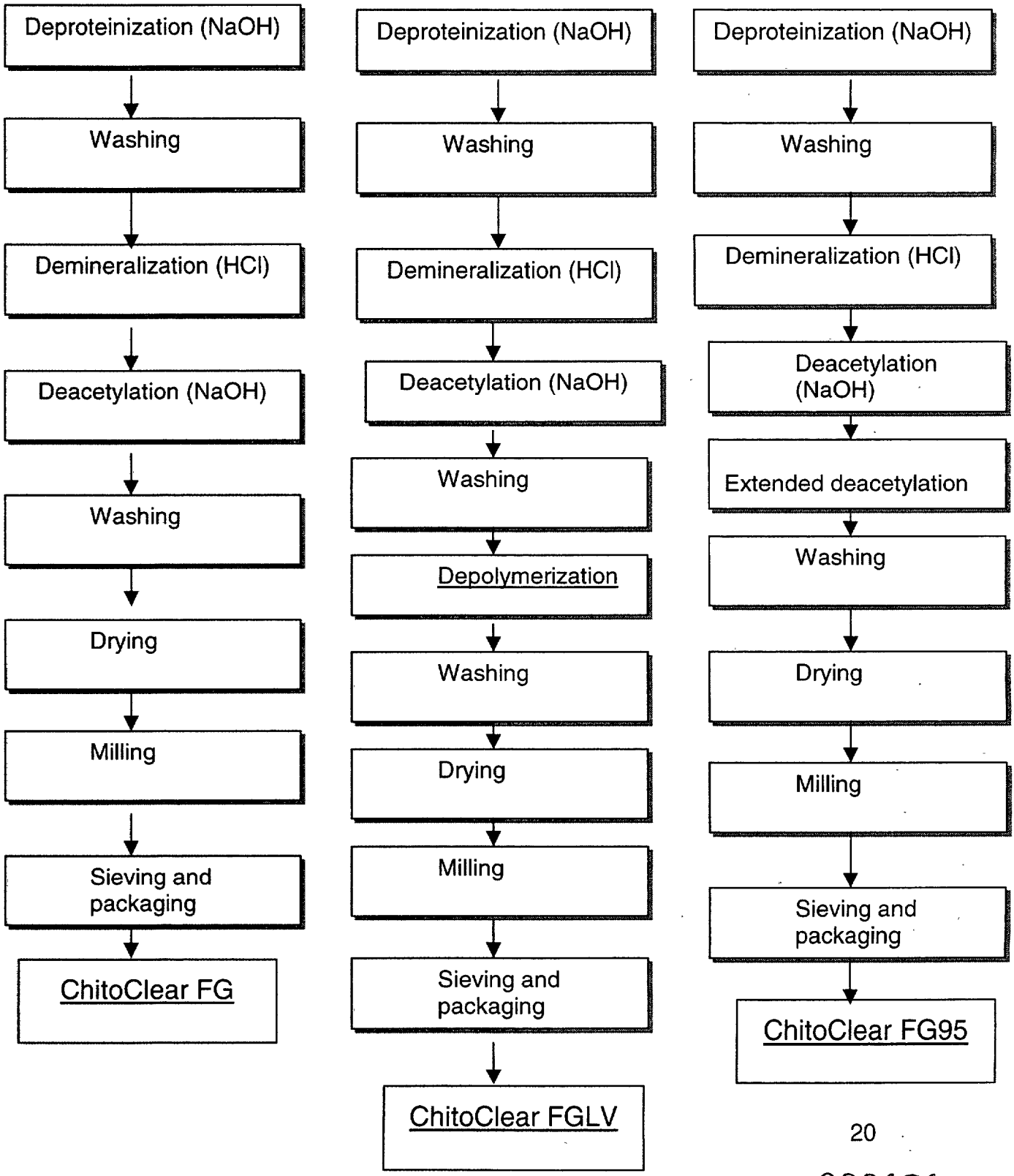
ChitoClear FG	Deproteinization, demineralization, deacetylation, drying/milling, sieving/packaging.
ChitoClear FG95	Deproteinization, demineralization, deacetylation, extended deacetylation, drying/milling, sieving/packaging.
ChitoClear FGLV	Deproteinization, demineralization, deacetylation, depolymerization, drying/milling, sieving/packaging.

Table 8
Primex Chitosan Products
Market and Properties

Primex Product Name	Properties of the Products	Market
ChitoClear FG	Highly deacetylated; Off-white flakes; Used in the general food market	General Use in Foods
ChitoClear FG95	Highly deacetylated; Off-white flakes; Used to absorb fat and to assist in maintaining healthy blood lipid level	Dietary Supplement
ChitoClear FGLV	Depolymerized; Off-white flakes; Low viscosity	Used in Foods to Maintain/improve emulsions.

Section II Chemical Identity of Chitosan

Figure 1
ChitoClear Product Specific Processing Steps



3. HACCP Standard and Recall Tracking Program

The entire chitosan production process at Primex Ingredients ASA is carried out according to the requirements of the HACCP standard. Written procedures are provided for each specification parameter. The various analyses listed in Tables 1-3 are performed on each lot of final product.

References for test methods:

1. Ash and dry matter content : SOP-04-019-03
2. Degree of deacetylation : SOP-04-020-02
3. Solubility : SOP-04-40-02
4. Turbidity : SOP-04-021-04
5. Viscosity : SOP-04-016-03
6. Heavy metal content : SOP-04-024-01
7. Microbiology : SOP-04-029,030,028,027,031,033-02

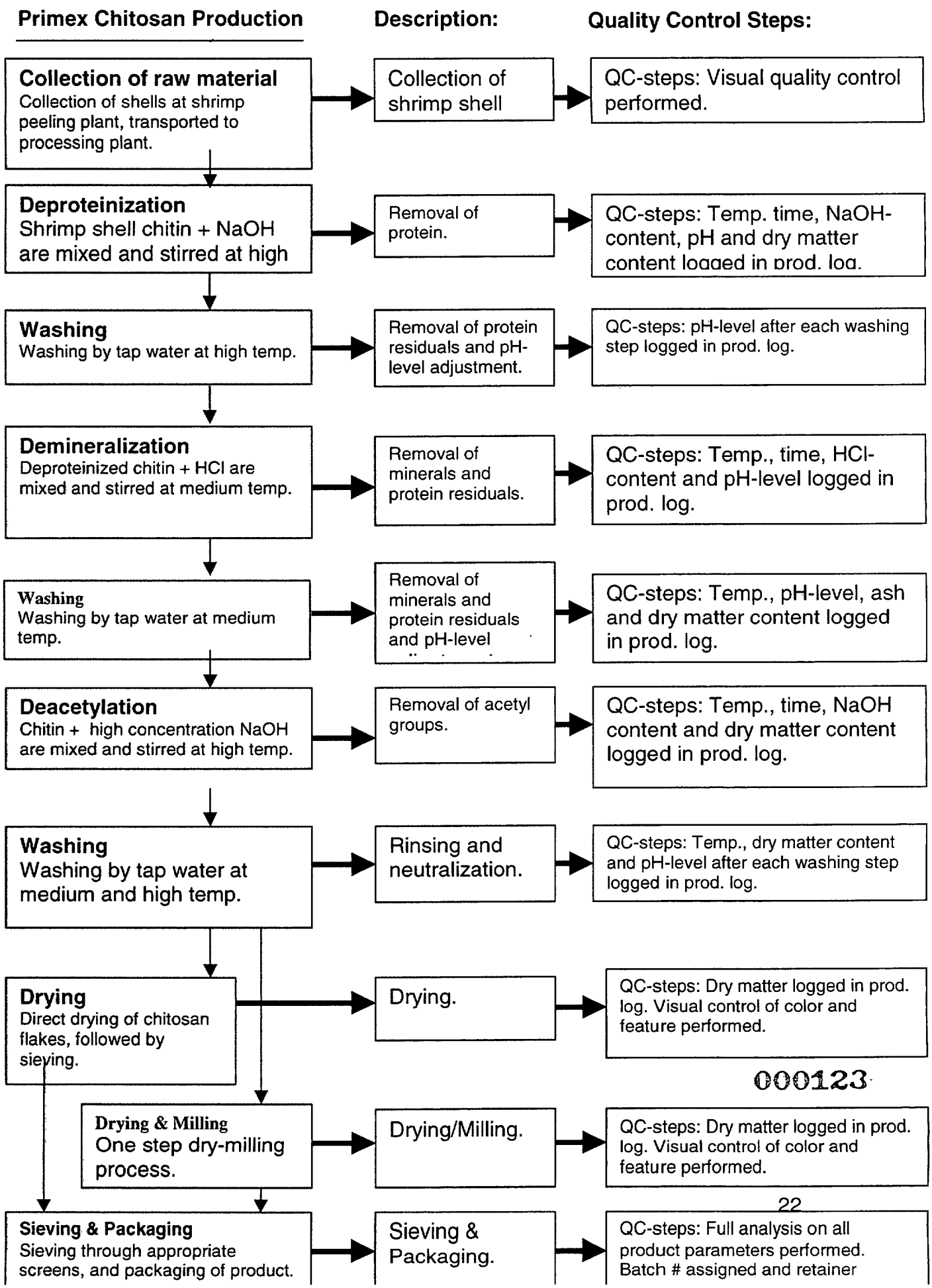
4. Critical Control Points in ChitoClear FG production:

The Primex Ingredients ASA chitosan plant has a certified Hazards Analysis Critical Control Point Program (HACCP) in place to assure product quality. Each segment of the Primex chitosan process is monitored for quality control using critical control points, including raw materials procurement and storage.

Analytical methods are used to ensure that the final product parameters remain within food grade specifications. [Primex Ingredients Analytical Methods Vol 7 Tab 118].

The critical control points of the Primex production process are listed below and are shown in Figure 2.

1. Receipt of raw material: Visual control of shell. Check for odor.
2. Receipt of production chemicals: Visual control of package.
3. Deproteinization: pH level, temp, time of stirring, NaOH content, dry matter.
4. Demineralization: pH-level, temperature, time of stirring, acid content.
5. Deacetylation: NaOH content, temperature, time of stirring, dry matter.
6. Washing after deacetylation: pH-level, temperature


Primex Chitosan Production
Description:
Quality Control Steps:
Collection of raw material
Collection of shells at shrimp peeling plant, transported to processing plant.
Collection of shrimp shell
QC-steps: Visual quality control performed.
Deproteinization
Shrimp shell chitin + NaOH are mixed and stirred at high
Removal of protein.
QC-steps: Temp. time, NaOH-content, pH and dry matter content logged in prod. log.
Washing
Washing by tap water at high temp.
Removal of protein residuals and pH-level adjustment.
QC-steps: pH-level after each washing step logged in prod. log.
Demineralization
Deproteinized chitin + HCl are mixed and stirred at medium temp.
Removal of minerals and protein residuals.
QC-steps: Temp., time, HCl-content and pH-level logged in prod. log.
Washing
Washing by tap water at medium temp.
Removal of minerals and protein residuals and pH-level
QC-steps: Temp., pH-level, ash and dry matter content logged in prod. log.
Deacetylation
Chitin + high concentration NaOH are mixed and stirred at high temp.
Removal of acetyl groups.
QC-steps: Temp., time, NaOH content and dry matter content logged in prod. log.
Washing
Washing by tap water at medium and high temp.
Rinsing and neutralization.
QC-steps: Temp., dry matter content and pH-level after each washing step logged in prod. log.
Drying
Direct drying of chitosan flakes, followed by sieving.
Drying.
QC-steps: Dry matter logged in prod. log. Visual control of color and feature performed.
Drying & Milling
One step dry-milling process.
Drying/Milling.
QC-steps: Dry matter logged in prod. log. Visual control of color and feature performed.
Sieving & Packaging
Sieving through appropriate screens, and packaging of product.
Sieving & Packaging.
QC-steps: Full analysis on all product parameters performed. Batch # assigned and retainer

000123

5. Primex tracking and recall program

Lot numbers are assigned to each batch produced from raw material, which follow the batch throughout the production procedure. New lot numbers are given in chronological sequence when the final product is obtained. These lot numbers start with the designation TM (abbreviation for "tørket/møllet" in Norwegian - Dried / Milled, example "TM-XXXX"). The relation between the two lot number systems is kept on record for a minimum of 3 years. Accurate record keeping on the basis of the lot numbers assures that the product and all raw materials can be tracked from receipt to the retail level.

Adequate size retainer samples are stored for a minimum of 3 years from production in order to provide material for testing if necessary. Therefore, Primex Ingredients assures that it has the programs in place to execute a limited or general recall, in the event that its quality control system is breached. This program is described in a separate SOP (01-001-00).

Recall Program

The Primex recall program is designed to operate as follows:

-Non-conformance is recorded on a non-conformance report.
-The customer is asked to send a sample of the lot in question.
-The retainer lot is checked according to Primex procedures.
-The lot is traced back to the raw material and all production logs, and any potential non-conformance reports are investigated. Personnel involved in the production of the lot in question are "interviewed" to resolve any discrepancies.
-The customer is contacted to coordinate procedures at their warehouse.
-The complete lot is recalled from all customers if necessary.

The Primex tracking and recall program is depicted below in Figure 3.

Figure 3
Primex tracking and recall program

Location		Documentation
1: Peeling factory	→	a: Log date and source of shrimp.
↓		
2: Raw material	→	b: Assign production lot number
↓		
3: Deproteinization	→	c: Record use of raw materials
↓		
4: Demineralization	→	d: Record use of raw materials
5: Deacetylation	→	e: Record use of raw materials
↓		
6: Drying / Milling		
↓		
7: Finished product	→	f: Retainer sample
		g: Assign final TM lot number

H. Characteristic Properties of Chitosan

1. Introduction

Comparison of the Physicochemcial Properties of Various Chitosans

Chitosan possesses certain physical properties, which form the basis for its functionality in foods. While the role of chitosan in food formulations will be discussed in Section II, several of the molecules basic properties are discussed below. Physicochemical characteristics and functional properties of five commercially available chitins and five chitosans were investigated by Shepard, *et al.,* 1997 [Shepard, *et al.,* 1997 Vol 8 Tab 146]. The physicochemical characteristics (nitrogen, ash, degree of deacetylation, bulk density, and viscosity) differed across the products tested. In functional properties, dye binding capacity differed depending on the products, although the average binding capacity (63%) of chitosans was higher that that (54%) of chitins [Shepard, *et al.,* 1997 Vol 8 Tab 146]. Water binding capacity ranged from 381 to 673% for chitins and from 458 to 805% for chitosans. Fat binding capacities of chitins were mostly similar (316-320%) except for one product (563%), whereas chitosans showed dissimilar binding capacities from 314 to 535%. However, the authors reported a significant correlation between water binding capacity and bulk density ($r = -0.89$, $P < 0.01$) and between fat binding capacity and viscosity ($r = 0.72$, $P < 0.05$) for chitin products. Both water and fat binding capacity of chitosan products were significantly correlated with ash ($r = 0.81$, 0.80) and with bulk density ($r = -0.98$, -0.95). The emulsifying capacity of egg yolk increased by addition of chitosan compared with the control. The authors stated that no differences in the emulsifying capacity of chitosan products were observed at any of the concentrations tested [Shepard, *et al.,* 1997 Vol 8 Tab 146].

2. Chitosan as an Absorbent of Free Fatty Acids in Palm Oil

Ahmad *et al.,* 1997 studied the sorption capacities of low molecular weight chitosan. The purpose of the study was to determine whether chitosan could absorb free fatty acids in oils, and thereby protect the oils from rancidity caused by the reaction of the free fatty acids with either oxygen or water. The authors' results showed that there are maximum sorption capacities of individual fatty acids towards chitosan; i.e., stearic (C_{18}:0), palmitic (C_{16}:0), myristic (C_{14}:0), oleic (C_{18}:1), and linoleic (C_{18}:2). Further, chitosans appeared to have a general pattern of absorption. Each fatty acid showed a maximum value of sorption to chitosan. The saturated fatty acids, which have

longer carbon chains showed higher sorption capacities than did unsaturated fatty acids. In this study, stearic acid (C_{18}:0) had a higher sorption capacity than the other fatty acids tested, approximately 18 mg/g of chitosan. The lowest sorption capacities were given by myristic acid (C_{14}:0). The total sorption capacities of saturated acids decreased in the following order: C_{18}:0 (stearic) > C_{16}:0 (palmitic) > C_{14}: 0 (myristic) [Ahmad *et al.,* 1997 Vol 3 Tab 2]. The author concluded that chitosan might be useful in the treatment of oils to prevent rancidity.

3. Physical and Chemical Structure of Chitosan

Chitosan, a poly-glucosamine, is a natural substance found in the cell walls of fungi, and in soil, and sediments [Gooday *et al.,* 1990 Vol 4 Tab 45 and Shimahara, *et al.,* 1989, Vol 9 Tab 154]. In nature most chitosan is a breakdown product from the degradation of chitin by certain groups of bacteria, which produce deacetylase or chitosanase enzymes. Commercially, chitosan is also produced by the deacetylation of chitin. Primex Ingredients ASA utilizes chitin from the Arctic shrimp *Pandalus borealis* as raw material for its chitosan production. Primex's commercial production of chitosan meets US HACCP standards, and results in a product, which is safe for human consumption and can be shown to be lower in lead and other contaminants than other chitosans, or other marine food products (See Tables 4, 5, 6, and 9).

Chitosan is a binary heteropolymer of ß (1→ 4)-2-amino-2-deoxy—D-glucopyranose and ß (1→ 4)-2-deoxy-2-acetamido-D-glucopyranose. It's raw material, chitin is the (1→ 4)-β-linked homopolymer of N-acetyl-D-glucosamine, and is one of the most abundant organic materials on the planet. The individual polymer chains of chitin appear to be helixes, since each sugar unit is inverted in comparison to adjacent sugar units [Gooday *et al.,* 1990 Vol 4 Tab 45]. This configuration may account for the great stability and unique characteristics of chitin and chitosan.

Primex Ingredients has developed a set of food grade specifications for its chitosan product, ChitoClear™, that defines a product, which contains ≥ 90% dry matter, less than 1.0 % ash, and negligible amounts of protein and fat. The percent deacetylation of Primex chitosan is greater than 70%, and the turbidity rating is ≤50 NTUs. The product is further characterized by a solubility of ≥98% in 1.0 % acetic acid. Lead levels are very low (<0.1ppm), and microbial limits have been set to meet food grade specifications (Tables 1-3).

Analyses of 5 lots in duplicate show that Primex is capable of meeting its specifications, and of producing a consistent product (See Table 4). Primex Ingredients has a product recordation system in place, and maintains retainer samples, in the event a general recall is necessary.

Various investigators have established that chitosan is capable of absorbing oils and fatty acids [Ahmad *et al.,* 1997 Vol 3 Tab 2]. Ahmad *et al.,* 1997 demonstrated that chitosan has a greater affinity for absorbing saturated fatty acids, than those with less saturation. Such physical characteristics appear to be of great usefulness in healthy foods.

I. Comparison of Primex Chitosan with Other Preparations

Table 9 shows a comparison between the specifications of commercial chitosans. It should be noted that Primex chitosan contains less lead, has a higher degree of deacetylation, and provides a lower limit on microbial content than do the other chitosans. This may be due in part, because of the different source organisms. Chitosan produced on the Pacific Rim is sourced from crab chitin, whereas that of Primex is sourced from arctic shrimp. It is logical to hypothesize, that the bottom-dwelling crab, would encounter more lead in its environment.

Table 9
Comparison of the Specifications of Commercial Chitosans

Variable	Japan	Korea	Kunpoong Bio	Primex
Description	White to bright yellowish	White to bright yellowish	White to bright yellowish	Off-White
Moisture	<12%	<15%	<7%	<10%
Deacetylation	>80%	>70%	>80%	>70%
Viscosity	>100 cPs	-	-	<5 cPs
Ash	<1.0%	<5.0%	<0.1%	<1.0%
Lead	<10 ppm	<20 ppm	<10 ppm	<0.1 ppm
CFU's/gram	<3,000			
Raw Material	Crab	Crab	Crab	Arctic Shrimp

[Kunpoong Bio Co., Ltd, Memo, April 1, 2000]

J. Chitosan in Nature

Section II Chemical Identity of Chitosan

Chitosan is a natural polysaccharide that occurs in nature in measurable amounts. It results in part from degradation of chitin from a variety of life forms (e.g. crustaceans) and from some species that produce chitosan directly. Chitosan is produced by several fungi, who utilize it in their cell walls as a structural component. Since there are vast amounts of fungi throughout nature with chitosan as an integral component, it is not surprising to learn that mankind has been unknowingly exposed to chitosan for millennia.

To illustrate this point, the fate of chitin in sediments in the Ythan Estuary, Aberdeen, Scotland has been studied by Hillman, Gooday, and Rosser (unpublished observations). The major input of chitin into this estuary is the productivity of the crustacean *Corophium volutator.* Adults of this species contain 8.4% chitin [Gooday *et al.,* 1990 Vol 4 Tab 45].

The annual input from this source alone was estimated as 3.7g chitin per square meter. Chitin and chitosan were detected in the sediments. Levels of both were reported to be considerably higher in winter than summer. Values for chitin and chitosan in the top 2 cm of sediments were 4.3 and 55.8 mg (g dry sediments)-1, respectively, in summer, and 36.2 and 156.7 mg g-1 in winter. Chitin contents declined rapidly with depth, being very low in the anaerobic sediment below 5 cm. Chitosan levels showed little variation with dept in the upper sediment, but were lower in the anaerobic sediment. It was suggested that much of the chitosan represented degradation of chitin produced in the sediment, whereas much of the chitin was from surface deposition. Chitinase and chitin deacetylase activities were detected in the sediment, both being higher in summer than winter. Values for chitinase were 93 and 31 mg chitin hydrolyzed hr-1 (g dry sediment)-1, respectively, for summer and winter; for chitin deacetylase, values were 33 and 5 mg chitin deacetylated h-1 g-1 for summer and winter. All assays were carried out at 150°C. Chitinase activities showed little variation in depth whereas chitin deacetylase activities were highest in the upper sediment. The finding of considerable levels of chitosan and chitin deacetylase suggest, that in these sediments a considerable amount of chitin degradation occurs via deacetylase, as well as by hydrolysis to chito-oligosaccharides, but the major agents of this deacetylation have yet to be identified. Litterbag experiments (nylon, 20-p.m mesh), using purified chitin, chitosan, and cellulose for comparison, gave rates of degradation for 2 months in the summer as 10.3, 7.0 and 4.4 mg (g polysaccharides)-1 day -1, respectively. Hillman *et al.,* (1989) reported the operation of a model system designed to imitate the natural estuarine environment and the author used it to study chitin

degradation. Results obtained were remarkably similar to those for the natural system; for example, relative rates for degradation of chitin =, chitosan, and cellulase for 1 month at 150°C were 2.4: 2.0:1, compared with 2.3:1.6:1 in the Ythan Estuary [Gooday *et al.,* 1990 Vol 4 Tab 45].

In nature chitosans are degraded by chitosanases, which have generally been recognized as enzymes that attack chitosan but not chitin. The Enzyme Commission defines chitosanase (EC 3.2.1.123) as an enzyme catalyzing the endo hydrolysis of β-1,4-glycosidic linkages between GlcNAc and GlcN residues in a partially acetylated chitosan. This kind of activity, however, was also observed at significant levels for enzymes first identified as chitinases, lysozymes, cellulases or hemicellulases, proteases, lipases, and β-1,3-1,4-glucanase. The Enzyme Commission's initial definition of chitosanases was accompanied by a comment that chitosanases "act only on polymers with 30% to 60% acetylation." The definition of this enzyme no longer holds, according to more recent findings on chitosanases. From the viewpoint of enzyme definition alone, further information about the structure and function of the chitosanase is needed [Gooday *et al.,* 1990 Vol 4 Tab 45]. Work at the Norwegian Biopolymer Laboratory and elsewhere has shown that there are many chitinases and chitosanases with specific but varying specificities for linkages and monomer sequences (O. Smidsrød, personal communication).

Table 10 below summarizes results from Shimahara, *et al.,* 1989, who studied the production of chitosan by various fungi in the family *Mucoraceae* [Shimahara, *et al.,* 1989, Vol 9 Tab 154]. The results show that certain species of fungi produced up to 180 mg/200ml of medium [Shimahara, *et al.,* 1989, Vol 9 Tab 154]. These data illustrate that chitosan is widespread in nature.

Section II Chemical Identity of Chitosan

Table 10
Amounts Of Alkali-Insoluble Materials (AIM) and Chitosan Produced By *Mucoraceae* Strains; Viscosities and Degrees of Acetylation of Chitosan Isolates

		AIM	Chitosan	%Chitosan/ AIM	Vis.
Ab.	*butleri Hut* 1001	416			16
				43	
Ab.	*California NRIC* 1165	352	137	39	9.4
Ab.	*Glauca NRIC* 1181	334	140	42	11
Ab.	*Glauca var. paradoxa NRIC* 1182				
		290	108	37	17
Ab.	*Japonica AHU* 9006	275	124	45	8.4
Ab.	*Orchidis HUT* 1034	328	163	50	17
Ac.	*Elegans IFO* 4022	295	35	12	NT
C.	*minor HUT* 1077	180	38	21	6.3
C.	*Mucoroides HUT* 1079	222	45	20	5.2
C.	*Muscae HUT* 1086	92	43	47	4.5
M.	*Alternans HUT* 1115	104	15	14	NT
M.	*dubius HUT* 1128	220	31	14	5.2
M.	*Guilliermondii IFO* 9403	408	30	7	NT
M.	*Inaequisporus IFO* 8624	35	13	37	NT
M.	*Tuberculisporus IFO* 9256	292	43	15	3.4
P.	*nitens IFO* 5694	46	13	28	Nt
			129		
R	*Acetoins*	330		39	12
R.	*Chinnensiss*		66		
		144	115	45	2.4
R.	*Nircinans*	212		54	5.1
R.	*Delemar*		84		
		292	98	29	2.4
R.	*Microsporous*	250	96	39	3.2
R.	*Nodosus*	274		35	2.6
Z.	*Californesis*	253	57	26	3.8
Z.	*Expoens*				
		466	65	14	1.6

Vis = Viscosity of 0.1 % chitosan solution (in 0.5 % acetic acid).
DA = Degree of acetylation evaluated from IR spectrum.
Ab. = Absidia ; Ac. = Actinomucor ; C. = Circinella; M. = Mucor ;
P. = Phycomycetes ; R. = Rhizopus; Z. = Zygorhynchus.
NT = Not tested.

K. Physicochemical Properties of Chitosan Which Form the Basis for Its Potential Functionality in Food

1. Introduction

Chitosan is a biocompatible cationic polysaccharide found indigenously in the cell walls of various fungi, crustaceans and insects [Illum, 1998 Vol 5 Tab 61]. It can also be chemically derived by deacetylating chitin, which is the second most abundant natural material on earth [Shahidi, *et al.,* 1999 Vol 8 Tab 145 and Shahidi *et al.,* 1998 Vol 8 Tab 144]. Chitin is similar to cellulose in structure, and is found, for example, in the exoskeletons of crabs and shrimps. The term chitosan refers to a family of polymers whose members differ in their degree of deacetylation (i.e., ratio of N-acetylated to deacetylated subunits) and molecular weight. These two characteristics are fundamental to the physicochemical properties of the chitosans and therefore have considerable influence on their biological activities [Illum, 1998 Vol 5 Tab 61 and Shahidi *et al.,* 1999 Vol 8 Tab 145].

Over the last decade, coinciding with the commercial production of higher purity chitosans, much interest and experimental activity has centered around the use of such chitosans in numerous food and biomedical applications. Chitosans have been found to have many beneficial properties of use to the food industry, including fat absorption, protein aggregation, emulsification capability, film-forming ability, clarifying ability and antimicrobial activity [Knorr, 1991 Vol 6 Tab 76 and Shahidi *et al.,* 1999 Vol 8 Tab 145].

This unique set of characteristics has led the chitosans to be considered as an ingredient in a wide array of food materials. Much of the evidence for the applicability of chitosan comes from the patent literature, where applications involving chitosan have ranged from edible films, to a component of artificial sweetening systems [U.S. Patent No. 5,741,505 VOL. 10 TAB 194, U.S. Patent No. 5,762,992, VOL. 10 TAB 195, and U.S. Patent No. 5,736,179 VOL. 10 TAB 193].

2. Potential Uses of Chitosan in Foods

Shahidi *et al.,* 1998 and 1999 summarizes the potential uses for chitosan in foods [Shahidi *et al.,* 1998 and 1999 Vol 8 Tabs 144 and 145]. The Table below was compiled by the authors.

Recent food application studies have shown that the addition of chitosan can contribute positive characteristics to baked goods

through emulsification (moistness and fineness of grain). However, the product appears to have a self-limiting level which, when exceeded produces a final consumer product, whose total quality is less than comparable controls [Poole, 1989 Vol 11 Tab 225]. Chitosan is currently being used in dietary supplement preparations as a source of dietary fiber, and as a hypocholesteremic agent [Primex Ingredients ASA Product Labels, Vol 8 Tab 126].

Section II Chemical Identity of Chitosan

Table 11
Food Applications of Chitin, Chitosan and Their Derivatives In The Food Industry

Area of application	Examples
Antimicrobial agent	Bactericidal Fungicidal Measure of mold contamination In agricultural commodities
Edible films	Controlled moisture transfer between Food and surrounding environment Controlled release of antioxidants Controlled release of nutrients, flavors and drugs Reduction of oxygen partial pressure Controlled rate of respiration Temperature control Controlled enzymatic browning In fruits Reverse osmosis membranes
Additive	Clarification and deacidification In fruits and beverages Natural flavor extender Texture controlling agent Emulsifying agent Food mimetic Thickening and stabilizing agent Color stabilization
Nutritional quality	Dietary fiber Hypocholesterolemic effect Livestock and fish feed additive Reduction of lipid absorption Production of single cell protein Antigastric agent Infant feed ingredient
Recovery of solid materials Food processing wastes	Affinity flocculation Fractionation of agar
Purification of water	Recovery of metal ions, Pesticides, phenols and PCB's Removal of dyes
Other applications	Enzyme immobilization Encapsulation of nutraceuticals Chromatography Analytical reagents

a) Chitosan as a Source of Dietary Fiber

The current definition of dietary fiber consists of the remnants of edible plant cell walls, including: polysaccharides, lignin, and associated substances resistant to (hydrolysis) digestion by the alimentary enzymes of humans [Dreher, 1987 Vol 4 Tab 27]. Many food scientists have considered chitosan a source of dietary fiber [Furda, 2000, Vol 4 Tab 40, and Dreher, 1987 Vol 4 Tab 27].

Dreher in the Handbook of Dietary Fiber, classified chitosan as a non-traditional source of dietary fiber, and stated that chitin/chitosan were aminopolysaccharides. Dreher described chitin as being similar to cellulose, except that it contained N-acetylamine groups at each 2-carbon atom of the glucose molecule [Dreher, 1987 Vol 4 Tab 27]. As stated above, chitin is the second most abundant organic compound on earth [Knorr, 1991 Vol 6 Tab 76], and is found especially in marine invertebrates, insects, fungi, and yeasts. Chitin represents 14-27% and 13-15% of the dry weight of shrimp and crab-processing wastes, respectively. It is a rather inert material and is very insoluble, however, the deacetylation of chitin with a strong base yields chitosan, which is a more soluble material, especially in dilute acids [Shahidi *et al.,* 1998 Vol 8 Tab 144]. According to Dreher, chitin and chitosan appear to have great potential in food science [Dreher, 1987 Vol 4 Tab 27].

Dreher speculated that chitosan might have similarities to microcrystalline cellulose, another dietary fiber, with a long history of use in the food industry. Microcrystalline chitin was thought to have suitable applications as a food thickener and stabilizer [Dreher, 1987 Vol 4 Tab 27]. Its viscosity and emulsion stability was reported to be 10 to 20 times higher than that of microcrystalline cellulose. Therefore, microcrystalline chitin might have application in foods such as peanut butter and mayonnaise. Knorr demonstrated that the addition of microcrystalline chitin to protein-fortified breads tended to increase specific loaf volume [Knorr, 1991 Vol 6 Tab 76]. This indicated that the surfactant properties of chitin might be of special interest to the baking industry. The addition of microcrystalline chitin decreased the water activity (AW) of bread crumb by 10%. Chitosan, due to its high viscosity in systems of acidic pH, was thought to be a good candidate for a food thickener [Dreher, 1987 Vol 4 Tab 27].

b) Source of D-Glucosamine

Today, one of the main uses of chitosan is as a feedstock or source for glucosamine. Hirano indicated in 1989 that approximately 60 tons of chitosan per year went into the production of D-glucosamine [Hirano, 1990 Vol 5 Tab 54].

Chitosan can function as a source of glucosamine, when taken as a dietary supplement. Glucosamine is an anti-inflammatory compound, synthesized in the human body from glucose and incorporated into gylcosaminolycans and glycoproteins [Muzzarelli, 2000 Vol 7 Tab 105].

Chitosan provides a sustained-release form of glucosamine. Oral ingestion (1g/day) of N-acetylglucosamine or chitosan increases the serum concentration of glucosamine [Muzzarelli, 2000 Vol 7 Tab 105]. With ingestion of chitosan, the serum N-acetylglucosamine concentration remains elevated for at least 48 hours, although glucosamine itself is normally cleared rapidly from the blood stream [Muzzarelli, 2000 Vol 7 Tab 105]. Blood glucose levels are not altered with supplemental N-acetylglucosamine. Setnikar *et al.*, 1993, indicated that these results confirmed the efficacy of orally administered chitosan for the improvement of joint function in subjects with osteoarthritis [Muzzarelli, 2000 Vol 7 Tab 105].

Experimental evidence from various laboratories indicates that orally administered chitosan is partially digested and absorbed [Muzzarelli, 2000 Vol 7 Tab 104 and Varum, *et al.,* 1997 Vol 11 Tab 213]. It is hypothesized that the digestion is due to the action of hydrochloric acid in the stomach, and the activities of nonspecific enzymes present in saliva and gastric juice. In the large intestine, enzymes from microorganisms further digest chitosan into glucosamine units [Muzzarelli, 2000 Vol 7 Tab 105].

Other foods have also been reported to provide a source of glucosamine. Tempe, a traditional Indonesian food, is prepared with cooked soya beans bound into a solid growth of *Rhizopus oligosporus.* The glucosamine content of this preparation is 51 - 107 g/kg (dry biomass). This example illustrates the fact that polymeric forms of glucosamine have been present in the human diet for generations in various foods [Muzzarelli, 2000 Vol 7 Tab 105].

The pharmacokinetics of glucosamine sulfate has been investigated after intravenous, intramuscular and oral administration in dogs, rats and humans. After intravenous administration of radio labeled glucosamine in dogs, investigators found that the substance was cleared from plasma in less than 2 hours and appeared to be incorporated into plasma proteins within a maximum period of 8 - 10 hours after injection. Nearly one third of the injected glucosamine could be recovered from the urine after 120 hours, and 1% was excreted in the faeces [Muzzarelli, 2000 Vol 7 Tab 105].

In human volunteers the data was similar. Upon oral administration, the concentrations of glucosamine were five times lower than after intravenous administration, probably because the glucosamine was removed in the liver, where it is metabolized to carbon dioxide, water and urea [Muzzarelli, 2000 Vol 7 Tab 105]. Glucosamine is known to diffuse very rapidly in most tissues and organs, even after oral administration, accumulating in the articular tissue and in the bone [Muzzarelli, 2000 Vol 7 Tab 105].

The clinical efficacy and safety of orally administered glucosamine has been surveyed in small animals. Over three thousand practitioners were asked to report on the effects of glucosamine treatment of degenerative joint diseases in dogs. Over 90% (96.8%) reported that dogs older than 5 years were the most responsive to treatment. The response to supplementation was estimated to occur within 4 weeks (83%). The glucosamine product was rated good or excellent in improving mobility and alleviating pain in the animals. No practitioner reported a worsening of pain or mobility [Muzzarelli, 2000 Vol 7 Tab 105].

Although the anti-inflammatory activity of glucosamine is 50 - 300 times lower than that of drugs, such as indomethacin, the toxity of the latter is 1000 - 4000 times greater, and therefore, the therapeutic margin favors dietary glucosamine. Its anti-inflammatory action appears to be related to mechanisms substantially different from those of non-steroidal drugs, which act primarily through inhibition of cyclooxygenase. Glucosamine is not an inhibitor of cyclooxygenase, and therefore, its effects are prostaglandin independent [Muzzarelli, 2000 Vol 7 Tab 105].

Glucosamine stimulates proteoglycan biosynthesis, and the

newly synthesized proteoglycans may stabilize cell membranes, resulting in an anti-inflammatory effect. Glucosamine also reduces the generation of superoxide radicals by macrophages and inhibits lysosomal enzymes [Muzzarelli, 2000 Vol 7 Tab 105]. Deal and Moskovitz, 1999, as reported by Muzzarelli list the numerous clinical trials (> 20), where glucosamine has been tested on over 3000 subjects. One of the most recent clinical trials was conducted by Laffler *et al.*, 1999, on 34 males from the U.S. Navy diving and special warfare community, who presented chronic pain and radiographic evidence of knee or low back diseases. Knee osteoarthritis symptoms were relieved as demonstrated by various assessments including a physical examination score (-43.3%) [Muzzarelli, 2000 Vol 7 Tab 105].

Thus it was concluded that the supplementation relieved symptoms of knee osteoarthritis, while a larger set of data was needed before comment could be made on spinal diseases. Houpt *et al.*, 1999 who investigated the efficacy of glucosamine on pain and disability, concluded that glucosamine hydrochloride benefits some subjects with knee osteoarthritis [Muzzarelli, 2000 Vol 7 Tab 105]. Muzzarelli, 2000 concluded that, in general, the studies discussed above demonstrated a favorable response by the body to glucosamine [Muzzarelli, 2000 Vol 7 Tab 105].

c) Use of Chitosan as a Dietary Supplement in the U.S.

Use of chitosan as a dietary supplement in the U.S. has been growing rapidly. According to Information Resources, Inc., approximately US $6 million has been sold by the three leading brands since February, 1999. This translates to approximately 240,000 kilograms of consumption, at an average wholesale price of U.S. $25 per pound. Actual usage may be closer to 500,000 kilograms per year, since the *Natural Products Insider, 1999 Buyer's Guide* lists 72 suppliers/distributors of the chitosan in the U.S.

Consumer use is mainly for the substance's hypocholesteremic activity, and for its use as a dietary source of glucosamine [National Toxicology Program, 2000 Vol 7 Tab 108]. Only one company manufactures chitosan in the U.S. Vanson Incorporated, Redmond, WA, has purchased a chitin and chitosan plant in Redmond, WA. The purchase was completed in a transaction with Natural Biopolymer and its

parent company, Natural AS, in Norway in 1999. However, the majority of high quality chitosan that meets consumer and biomedical specifications (See Section I) is imported from suppliers outside the U.S.

d) Usage of Chitosan in Japan

The Japan Times of April 11, 2000 reports that sales of dietary chitosan in Japan totaled total nearly US$1 billion per year [*Japan Times,* April 11, 2000 Vol 7 Tab 108]. However, a 1999 U.S. State Department Document entitled "Japan – Chitin and Chitosan Market – IMI990408" indicates that the current health food market for chitosan in Japan is slightly more than 200 metric tons per year. Total chitin production for the same period was estimated at 1,000 metric tons per year [U.S. State Department Report IMI990408, 1999 Vol 7 Tab 108]. Further, Japan exports approximately 200 MT of chitosan per year to the U.S. and Europe. Current prices for chitosan for food use were reported to be 4,000 – 5,000 yen/kilo (US $33.33 to 41.66 per pound, when 120 yen = US$1.00). This makes chitosan a very expensive ingredient in the Japanese market. According to the State Department Report, the total market for chitosan has dropped, due to competing products in the wastewater sector [U.S. State Department Report IMI990408, 1999 Vol 7 Tab 108]. According to the *Korea Times* nearly 50% of the chitosan manufactured in Japan is used in purifying drinking and wastewater streams [*Korea Times*, April 7, 1999 Vol 12 Tab 236].

The *Korea Times* also reports that Japan is the world's leader in the manufacture of chitosan, accounting for 90% of the global chitosan market worth approximately 4 trillion yen (120 yen = 1 US dollar) [*Korea Times*, April 7, 1999 Vol 12 Tab 236]. The report indicated that there are ten firms manufacturing chitosan in Japan, and that 60-package chitosan into various products [*Korea Times*, April 7, 1999 Vol 12 Tab 236].

Currently, certain foods containing chitosan have been approved in Japan as Foods for Specific Health Use (FOSHU). Those foods include a biscuit (Healket™) by Nippon Kayaku Co., Ltd., a fish cake (Health Support Food "Marin" Including Chitosan™) by Ito Han Foods, Inc., and a biscuit from Nippon Menard Cosmetic Co., (Menard CholeToru Bar™). All three

foods carry labeling for cholesterol reduction [FOSHU, 1999 Vol 7 Tab 108].

Other foods containing chitosan include: "Oppen Bifidus Chitosan" by Oppen Cosmetics. The product is a health food supplement, which is designed to maintain a healthy gastrointestinal tract. It contains chitosan which cleanses the intestines, lactic acid bacteria (*Bifidus bacteria* and *Lactobacillus faecalis*), which regulate the function of the intestines, "Kohki [*Engelhardtia chrysolepsis*] Extract", which decreases neutral fat and suppresses obesity, raffinose, which is a nutritional source for *Bifidus bacteria*, collagen, which is a substance reputed to be an effective skin moisturizer, and B group vitamins. The product is formed into a small triangular tablet, and has "an elegant prune flavor", according to the manufacturer. A package of 210 tablets costs Y5500 (US$ 45.83) [*Japanese Cosmetic and Household Goods Monitor*, 1999 Vol 8 Tab 126].

e) Chitosan Use in China

Qindao Golden Yuan Biological Products Co. Ltd. has indicated in its product literature, that it is the largest chitosan manufacturer in China [Qindao Golden Yuan Biological Products Co. Ltd., 1999 Vol 8 Tab 130]. The company reports its plant capacity at 200 metric tons, most of which is exported to the United States and Europe. The product is derived from the Alaskan crab, and its uses in China according to a company representative are similar to those in Japan.

3. Mechanism of hypocholesterolemic and lipid lowering effects of chitosan

Chitosan has been cited for its ability to reduce cholesterol levels [Muzzarelli, 2000 Vol 7 Tab 105]. While various mechanisms have been suggested for this effect, it may be important to consider cholesterol metabolism in general in order to assess the functionality of chitosan for this purpose.

a) Digestion and absorption of Cholesterol

Cholesterol is the most abundant sterol in mammalian cells. It is required for normal cell growth and proper membrane structure and function. The brain contains 10% cholesterol (dry weight), most of which is incorporated into the myelin

sheaths of neurons. The liver is the primary organ for the maintenance of cholesterol homeostasis in the body. Adrenals, gonads and placenta also transform cholesterol into hormones [Muzzarelli, 2000 Vol 7 Tab 105].

The most important elimination pathway for water-insoluble cholesterol is its conversion to water-soluble bile acids. The flux of cholesterol and bile acids regulates the activity of the following enzymes: acylcoenzyme A transferase (ACAT); cholesterol 7-alpha-hydroxylse (a monooxygenase located in the endoplasmic reticulum); 3-hydroxy-3-methylglutaryl-coenzyme A reductase (hmg-CoA-R). Bile acids are efficient suppressors of the latter enzyme, HMG-CoA-R, in vivo [Muzzarelli, 2000 Vol 7 Tab 105].

Unregulated accumulation of cholesterol is cytotoxic and a failure to maintain homeostasis of the sterol results in a number of pathologies, such as gallstone disease, atherosclerosis, corneal crystalline dystrophy, and tumor proliferation [Muzzarelli, 2000 Vol 7 Tab 105]. There is therefore, much interest in keeping cholesterol levels within healthy boundaries, and in limiting its ingestion.

The human body reacts to interventions intended to lower cholesterol concentration. The effect of cationic resins on cholesterol degradation is counteracted by a compensatory increase in cholesterol synthesis. For instance, biliary diversion or administration of bile acid-binding cationic resins leads to a several-fold stimulation of both bile acid biosynthesis and cholesterol 7-alpha-hydroxylase activity [Muzzarelli, 2000 Vol 7 Tab 105].

Cholesterol is generally ingested with fat. The process of fat digestion begins in the stomach, with a fraction of the triacylglycerols being hydrolyzed by the pre-duodenal lipases. Lipases are key enzymes in the hydrolysis of triacylglycerols, phospholipids, and cholesteryl esters, including those of dietary origin. Their actions are essential to maintain lipid homeostasis and cardiovascular health [Muzzarelli, 2000 Vol 7 Tab 105].

Dietary cholesterol is delivered to the intestinal lumen as crude lipid emulsion particles with the undigested trigylcerols and phospholipids. Within the lumen of the intestine, triacylglycerols, phospholipids and cholesteryl esters in the

emulsion are hydrolyzed further by pancreatic enzymes, including pancreatic lipase, phospholipases and cholesterol esterase. The digested products are mixed with phospholipids and bile salts in micellar structures, or complexed with phospholipids in vesicles [Muzzarelli, 2000 Vol 7 Tab 105].

The products of triacylglycerol hydrolysis by pancreatic lipase and colipase, namely monoacylglycerol and free fatty acids, are key determinants in mediating cholesterol transport from lipid emulsions to intestinal cells. The surface and core components a lipid carries are crucial for absorption of dietary cholesterol in the gastrointestinal tract. Cellular uptake of cholesterol from emulsions with a phospholipid / triacylglycerol molar ratio < 0.3 could be stimulated by pancreatic lipase and colipase hydrolysis of the core neutral lipids. However, the enzyme is ineffective in emulsions with a phospholipid triacylglycerol molar ratio > 0.3 [Muzzarelli, 2000 Vol 7 Tab 105].

The inhibition of triacylglycerol digestion thus impacts on the absorption of dietary cholesterol. The latter, which exists in the intestinal lumen in emulsion particles with phospholipids and triacylglycerols may be absorbed via different mechanisms than is biliary cholesterol, which is primarily associated with bile salt mixed micelles [Muzzarelli, 2000 Vol 7 Tab 105].

b) Absorption of Dietary Cholesterol

According to Muzzarelli, 2000, new light has been shed on the absorption process of dietary cholesterol in the course of studies on small intestine brush border receptors. The following three processes promote absorption of the water-insoluble lipids into intestinal epithelial cells (enterocytes):

i. Hydrolysis of triacylglycerols and phospholipids by pancreatic lipase; lipids and hydrolysis products are dispersed in the lumen of the small intestine by bile salts yielding micelles and vesicles, which transport lipids via Brownian diffusion to the surface of the small intestine.

ii. The lipids are transferred into the brush border membrane of enterocytes, by protein-mediated collision-induced transfer.

iii. A simple diffusion mechanism is effective when the solubility of the lipid in water is above a certain level (≥ 10 M for monoacylgylcerols), but it does not work for water insoluble lipids such as cholesterol and cholesteryl esters (≤ 10 M) (Hauser *et al.*, 1998), as referenced in [Muzzarelli, 2000 Vol 7 Tab 105].

Muzzarelli, 2000 lists other mechanisms important in cholesterol transport:

i. Cellular lipoprotein uptake, i.e. internalization of exogenous cholesterol, can occur via receptor-mediated endocytosis.

ii. LDL binds tightly to the 20,000 - 50,000 receptors on each cell. After binding LDL-cholesterol is delivered to the metabolically active pool of cholesterol inside the cell.

iii. Unlike LDL, the protein component of HDL is not degraded in the transport of cholesterol. The HDL binds to the cell, and the lipid-depleted particle is released into the extracellular space.

iv. Dietary cholesterol is initially in the form of free cholesterol and cholesteryl esters: the latter is hydrolyzed by pancreatic cholesteryl ester hydrolase. Biliary cholesterol is entirely in the form of esters. On entering the mucosal cell, cholesterol becomes esterified and packaged into chylomicrons, which are transported through the lymphatics into the circulatory system. Sterol uptake by mucosal cells is relatively specific for cholesterol. [Muzzarelli, 2000 Vol 7 Tab 105].

v. The scavenger receptor SR-BI is located in the brush border membrane of enterocytes and assists the uptake of dietary sterols and other classes of lipids. Hauser *et al.*, 1998 demonstrated that SR-BI is active in facilitating lipid uptake, for instance cholesteryl oleate from egg phosphatidylcholine vesicles into brush border membrane vesicles. The kinetics of cholesterol uptake by caco-2 cells were studied in the presence and absence of anti-human SR-BI IgG, and evidence for

SR-BI being inhibited by specific ligands that prevent cholesterol absorption was provided [Muzzarelli, 2000 Vol 7 Tab 105].

vi. According to Muzzarelli, the fact that the receptor protein works when the lipid is correctly accompanied by bile salts, and that it could be inhibited by certain compounds, might indicate that it could be relatively easy to interfere with the correct interaction of cholesterol with the docking receptor, and to inactivate that receptor [Muzzarelli, 2000 Vol 7 Tab 105].

c) Proposed Mechanisms for the Hypocholesterolemic and Lipid Lowering Effects of Chitosan

Furda, 2000 has reviewed the proposed mechanisms, which have been thought to explain the hypocholesterolemic and lipid lowering effects of chitosan [Furda 2000, Vol 4 Tab 40]. These include:

i. Viscosity effect
ii. Ionic interaction
iii. Entrapment of mixed micelles and bile acids and fatty acids
iv. Entrapment of dietary triglycerides
v. Inhibition of pancreatic lipase

Each of these effects will be discussed below.

i. Viscosity effect

It has been believed that chitosan, in a similar manner to other viscous polysaccharides, can reduce the absorption of cholesterol and bile and fatty acids by acting as a physical barrier to the diffusion of nutrients across the intestinal wall. Chitosan is believed to coat the inner walls of the small intestine [Vahouny 1982, Vol 11 Tab 211, and Ikeda *et al.*, 1989 Vol 5 Tab 60]. Unlike other polysaccharides, however, chitosan is viscous only under conditions of low pH. In the small intestine, at neutral pH, chitosan gradually precipitates, loosing its viscous character. Lacking significant viscosity in localities where absorption of nutrients takes place, the gastric viscosity of chitosan may play only a minor role in reducing the absorption of lipids and

cholesterol. In the case of cholesterol, this assumption is supported by results from a study with chitosan preparations having a comparable degree of deacetylation but differing widely in viscosity (17- 1,600 cP). In this study all preparations almost completely prevented the rise of serum cholesterol in rats fed a cholesterol-enriched diet containing 2-5% chitosan [Sugano *et al.*, 1988 Vol 9 Tab 156].

The fact that a large number of studies, with animals or humans demonstrated the hypocholesterolemic effects of chitosan, in which different preparations of chitosan with different viscosities were used, suggests that this parameter may not be of critical importance for achieving significant cholesterol lowering effects.

Interestingly, even chitosan hydrolyzates having viscosities as low as 1.6 cP showed hypocholesterolemic and lipid lowering effects in the serum and liver of rats fed a cholesterol enriched diet. The effects were equal in their magnitude to unhydrolyzed chitosan [Ikeda *et al.*, 1993 Vol 5 Tab 60].

Partially hydrolyzed chitosans with average molecular weights of 8.0×10^3 and 2.0×10^4 had equal or slightly greater cholesterol lowering potential than unhydrolyzed chitosan with a molecular weight of 3.0×10^5. Extensively hydrolyzed chitosans, however with molecular weights lower than 5.0×10^3 and viscosities lower than 1.3 cP, including glucosamine oligomers of DP 2-7 lacked cholesterol and lipid lowering effects in the serum and liver of rats fed a cholesterol enriched diet [Sugano *et al.*, 1992 Vol 9 Tab 158 and Ikeda *et al.*, 1993 Vol 5 Tab 60].

Furda, 2000 summarized that, maximum cholesterol lowering was observed with chitosan preparations having viscosities of 1.6-1.9 cP and average Mw 10^4 - $2x10^4$. Preparations with molecular weights of 70 kDa and 750 kDa were more effective plasma cholesterol reducers, than chitosan with molecular weight of 2,000 kDa [Lehoux *et al.*, 1993 Vol 6 Tab 93]. In a study conducted by Lehoux *et al.*, 1993, the author observed that chitosan, unlike cholestyramine, decreased the activity of HMG-coA reductase in rat diets containing

5% chitosan and 1% cholesterol. At the 7.5% level, chitosan maintained adequate cholesterol homeostasis in rats, despite a greatly increased intake of cholesterol. A study by Lian *et al.*, 1998 also showed that low and medium viscosity chitosans (100 mPas and 200 mPas) were somewhat more effective than high viscosity chitosan (400 mPas) in serum cholesterol reduction in rats [Furda 2000, Vol 4 Tab 40].

Effect on dietary lipid absorption

Deuchi *et al.,* (1995) have shown that as the *in vitro* viscosity of chitosan preparations having comparable degrees of deacetylation increased, the apparent fat digestibility by rats fed a high fat diet decreased. While the apparent fat digestibility in the presence of chitosans with viscosities of 5 -10 cP was 70%, the fat digestibility in the presence of chitosan with viscosities of 450-500 cP was 50%. This result was unexpected for two reasons. First, as mentioned above, chitosan precipitates in the small intestine at neutral pH, loosing its gastric viscosity near absorptive sites. Second, Kanauchi *et al.*, (1995) attributed the enhancing effects of ascorbic acid on the reduction of fat absorption by chitosan to the reduction of its gastric viscosity [Kanauchi, *et al.*, 1995 Vol 5 Tab 69]. It is possible, however, that the precipitation of chitosan in the small intestine (duodenum) of rats due to a gradual increase in pH is not necessarily spontaneous but rater gradual, and that chitosan remains viscous in the initial segments of the duodenum [Furda, 2000 Vol 4 Tab 40]. This apparently affects the fat absorption mechanism in rats more than the cholesterol absorption.

While the intestinal cholesterol absorption in lymph-cannulated rats was reduced by low viscosity chitosan hydrolysates as well as by vicious chitosan, the absorption of oleic acid and triglycerides was also reduced by viscous chitosan, but only delayed by chitosan hydrolysates [Vahouny *et al.*, 1983 Vol 11 Tab 212 and Ikeda *et al.*, 1989 Vol 5 Tab 59]. This again indicated that effective reduction of lipid absorption by chitosan, unlike reduction of cholesterol absorption, might require higher initial viscosity in the small

intestine, particularly in the duodenum [Furda, 2000 Vol 4 Tab 40].

In broiler chickens Razdan *et al.*, 1996, found that high viscosity chitosan at 1.5% in the diet, was marginally more effective in reducing the ileal crude fat digestibility, while the effect of low, medium and high viscosity chitosans on total plasma cholesterol and HDL cholesterol was in general favorable, but not conclusive in relation to their *in vitro* viscosity [Razdan *et al.*, 1996, Vol 8 Tab 134].

ii. Ionic interaction

Most of the early papers describing serum cholesterol reduction in experimental animals fed a chitosan-containing diet, suggested that ionic interaction between chitosan and bile acids could be responsible for this effect [Sugano *et al.*, 1978 Vol 9 Tab 155, 1980 Vol 9 Tabs 157; Nagyvary *et al.*, 1979 Vol 7 Tab 107, and Kobayashi *et al.*, 1979 Vol 6 Tab 77]. By binding bile acids and cholesterol, chitosan could disrupt micelle formation in the intestine [Sugano *et al.*, 1978 Vol 9 Tab 155]. This assumption was supported by others, who postulated that ionic interaction between the protonated amino groups of chitosan and negatively charged bile acids and fatty acids leads to enhanced excretion not only of bile acids and cholesterol, but of whole mixed micelles containing other lipids such as fatty acids, mono glycerides and phospholipids. The binding and excretion of bile acids results in a decreased cholesterol pool [Furda, 2000 Vol 4 Tab 40].

The existence of an ionic interaction between positively charged amino groups of chitosan and negatively charged bile acids or fatty acids is further supported by works of Ishi *et al.*, (1986) and Kanauchi *et al.*, (1995), who reported that chitosans with greater degrees of deacetylation and comparable viscosities were more effective in cholesterol lowering and fat absorption reducing action in rats than chitosans deacetylated to a lesser degree [Furda, 2000 Vol 4 Tab 40].

Binding of mixed micelles, bile acids, and fatty acids

Since chitosan is a weak base (pK = 6.5), it can bind different anions. The critical anions during lipid digestion are free fatty acids and bile acids. Even though the digestion of lipids is initiated in the stomach, in the gastric environment. (pH 1-3), chitosan is fully protonated. The amount of bound fatty acids, however, would be extremely small due to the low activity of gastric lipase and suppressed ionization of released fatty acids, which would be entirely solubilized in triglycerides during that stage of digestion [Furda, 2000, Vol 4 Tab 40 and Glickman, 1984 Vol 4 Tab 44].

Intestinal binding

It had been thought, however, that the majority of binding to fatty acids and bile acids takes place in the small intestine, namely in the duodenum and possibly jejunum [Nagyvary *et al.*, 1979 Vol 7 Tab 107, Sugano *et al.*, 1978 Vol 9 Tab 155, and Furda, 1983 Vol 4 Tab 39]. There, the remaining protonated amino groups of chitosan attract the negatively charged bile acids and fatty acids which are present in mixed micelles; the product of triglyceride emulsification and hydrolysis. The result of this attraction can lead to the attachment of the entire micelle to the chitosan chain, or to the disruption of the micelle. Based on their relative pK values, it could be expected that the bile acids, which are fully ionized at neutral pH, would be bound more extensively than the fatty acids, which are only partially ionized [Furda 2000, Vol 4 Tab 40].

Attachment of entire mixed micelles to chitosan chains could lead to their co-excretion with chitosan, provided that this attachment/binding was strong enough to withstand the process of digestion. Similar malabsorption of lipids would result from disintegration of mixed micelles. The ionically bound bile and fatty acids would be co-excreted with chitosan as well, provided that the bonds were sufficiently strong. Additionally, the insolubilized cholesterol and 2-monogylcerides would also be excreted after being expelled from the mixed micelles.

Since the powerful bile acid binders such as cholestyramine do not cause significant fat malabsorption or excretion of neutral sterols, it its not likely that chitosan, which is a weaker base, would cause disintegration of mixed micelles as a result of excessive binding of bile acids.

The studies of Sugano *et al.*, (1988, 1992) and Ikeda *et al.*, (1993), in which they studied the hypocholesterolemic action of chitosan hydrolysates and chitosan oligomers in rats, however, does not support the mechanism discussed above. The chitosan hydrolysates with an average molecular weight of 2,000 or less, did not show any serum cholesterol reduction, neither did they enhance excretion of acidic nor neutral steroids. Therefore, this mechanism alone, although plausible, may not fully explain the hypolipidemic properties of chitosan. Intestinal binding could, however, represent the initial phase of binding, followed by precipitation of chitosan, which leads to the entrapment of lipids and cholesterol [Furda 2000, Vol 4 Tab 40, Sugano *et al.*, 1988 Vol 9 Tab 156, Sugano *et al.*, 1992 Vol 9 Tab 158, and Ikeda *et al.*, 1993 Vol 5 Tab 60].

iii. Entrapment of mixed micelles, bile acids and fatty acids

While the ionic interaction of chitosan with negatively charged lipids is understandable from a theoretical point of view, the interaction with neutral lipids is more difficult to explain. Assuming that bile acids and fatty acids are bound by ionic linkages to chitosan, it could be expected that neutral lipids and cholesterol would be solubilized in the hydrophobic chain of fatty acids and ultimately co-excreted. However, since the primary binding between chitosan and bile and fatty acids may be neither extensive nor strong enough, Furda, 2000, questioned whether secondary hydrophobic binding could explain the reduced absorption and increased excretion of neutral lipids [Furda 2000, Vol 4 Tab 40].

After ingestion, chitosan is partially or fully solubilized and converted into a viscous liquid by gastric hydrochloric acid. As it enters the duodenum, it starts to precipitate under the influence of the neutral pH. This

precipitation is likely gradual because of the pH gradient in the duodenum (pH 5.5 - 7.5). It is therefore likely that the previously attached mixed micelles or bound bile and fatty acids will become entrapped in the chitosan chains during aggregation and precipitation in the duodenum [Furda 2000, Vol 4 Tab 40].

Being entrapped, the various lipid moieties, including micelles, bile acids and fatty acids, or free cholesterol and monoglycerides, will escape the absorption from the small intestine. This type of immobilization of lipids and their components between chitosan chains results in stronger bonding, which according to Furda, 2000, could withstand the process of intraluminal digestion [Furda 2000, Vol 4 Tab 40].

In support of this argument, it has been repeatedly reported that chitosans with degrees of acetylation between 5 - 30% and average molecular weights equal to or greater than 5,000 precipitate firm neutral solutions [Ikeda, 1993 Vol 5 Tab 60].

iv. Entrapment of dietary triglycerides

Kanauchi *et al.*, 1995, while studying the inhibition of fat digestion by chitosan and the synergistic effect of ascorbic acid and sodium ascorbate in rats proposed a mechanism for the inhibition of fat digestion that starts in the gastric environment. In the stomach chitosan solubilized by gastric acid emulsifies fat droplets by mixing with them, and then surrounding them [Kanauchi *et al.*, 1995 Vol 5 Tab 70].

After this "rough" emulsion reaches the small intestine, the solubilized chitosan changes into an insoluble gel, and the firmly entrapped fat droplets cannot be attacked by pancreatic or intestinal enzymes. Some fat droplets, which are emulsified by casein, are partly digested by pepsin, and those, which are not sufficiently emulsified by chitosan, would be expected to be digested in the intestine.

To follow the fate of ingested lipids, the corn oil, which was used in the study by Kanauchi, was stained with

red dye, and the red oil droplets were readily observable in the ileal contents of the test animals. This observation supported the theory, which suggested that a significant portion of corn oil was effectively emulsified and engulfed by chitosan in the stomach of the animals. This oil later became firmly entrapped as chitosan formed an insoluble gel during its precipitation in the small intestine. Although the authors did not consider the possibility that emulsified lipid droplets underwent enzymatic hydrolysis, it is possible that some or most of them did, and that the red dye remained solubilized and entrapped in the fatty acid. Although in Kanauchi's model, there is chitosan, which is not involved in the entrapment, no specific activity is attributed to it [Kanauchi *et al.*, 1995 Vol 5 Tab 70].

It is evident that the major requirement for the excretion of dietary triglycerides in Kanauchi's model is effective emulsification, and the ability of chitosan to engulf lipid droplets in the gastric environment. Generally, it is known that lipids do not emulsify easily in the stomach and they typically form a separate layer [Kanauchi *et al.*, 1995 Vol 5 Tab 70].

The precise mechanism of the emulsifying properties of chitosan are not clear, however, much depends on the conditions and presence of other ingredients. Knorr, 1982, had reported that chitosan was not an effective emulsifier for neutral oil *in vitro*. In spite of these concerns, it is possible that under certain conditions and in certain species the entrapped and excreted lipids are primarily or exclusively triglycerides containing dietary cholesterol as proposed by Kanauchi *et al.*, 1995 [Furda, 2000 Vol 4 Tab 40 and Kanauchi *et al.*, 1995 Vol 5 Tab 70].

v. Inhibition of pancreatic lipase

It has not been reported that chitosan inhibits the activity of human pancreatic lipase per se, which, if that were the case, would reduce the hydrolysis of dietary triglycerides and thus lead to their malabsorption. It was reported, however, that chitosan is partially hydrolyzed *in vitro* by different lipases, and is also slightly degraded by human amylase [Muzzarelli *et al.*,

1997 Vol 7 Tab 104]. The authors implied that if chitosan were introduced with the diet, it would in part prevent lipases from hydrolyzing the lipids. Whether lipases from the gastrointestinal tract of humans can effectively hydrolyze chitosan has not been conclusively shown.

4. Mechanistic alternatives

Three major pathways for the role of chitosan have been suggested, and these routes differ in a few specific points. Pathway 2A as outlined by Furda, 2000 assumes that dietary lipids are not emulsified and necessarily surrounded by chitosan chains in the stomach, but rather, they are coated by trace amounts of fatty acids released from the triglycerides by gastric lipase [Furda, 2000 Vol 4 Tab 40]. As the lipids enter the duodenum, the surface fatty acids become partially ionized, which initiates polar attraction between partially protonated chitosan chains and lipid droplets. The rapid precipitation of chitosan precedes the emulsification of lipids and the attack by pancreatic lipase, resulting in the entrapment of attracted lipid droplets without their undergoing enzymatic hydrolysis.

The end products of Pathways 1 and 2A are the same. Pathway 2B is identical with Pathway 2A in the gastric stage. In the intestine, however, the enzymatic hydrolysis of lipids precedes the precipitation of chitosan. The formation of mixed micelles is attracted by partially protonated chitosan chains, and is either immediately entrapped by precipitating chitosan (polar entrapment) or disintegrates prior to the individual components of the formation becoming entrapped by precipitating chitosan chains.

Under Pathways 1 and 2A, which exclude the possibility of enzymatic hydrolysis prior to lipid entrapment, one can largely expect the entrapment and excretion of dietary triglycerides and cholesterol, since the protonated amine groups of chitosan are likely to be involved in polar interactions with surface fatty acids. This makes them less available for other anions [Furda, 2000 Vol 4 Tab 40].

Under Pathway 2B, primarily acid steroids (bile acids) and also fatty acids should be excreted. Sugano *et al.*, 1978, and 1980, have shown that when rats were fed chitosan and cholesterol containing diets, the fecal output of neutral sterols, but not acid sterols, was increased. Fukada *et al.*, 1991 observed that the excretion of bile acids was not increased, but that their compositional profile was changed when male rats were fed chitosan-containing diets for three

weeks. Kanauchi *et al.*, 1995, after using dietary oil stained with red dye and observing the red oily droplets in the ileal contents of rats fed chitosan and oil containing diets, concluded that chitosan entrapped the droplets of dietary oil. They did not indicate or show entrapment, binding or excretion of bile acids. On the other hand, Ebihara *et al.,* 1989 demonstrated significantly increased concentrations of bile acids in the intestinal contents relative to the aqueous phase in rats fed test meals containing 5% chitosan or cellulose. Since excretion analysis was not conducted, it is not clear whether the bile acids were co-excreted with chitosan or reabsorbed. It is possible that some bile acids are weakly bound to chitosan in the upper intestine, which could destabilize mixed micelles, but these bile acids are released later and reabsorbed from the lower intestine [Furda, 2000 Vol 4 Tab 40].

From these experiments, it appears that in rats, the hypocholesterolemic and lipid lowering effects of chitosan may not be due to increased binding and excretion of bile acids. If there is any elevation of excretion of bile acids in rats fed chitosan hydrolysates in cholesterol containing diets, it is minor [Ikeda *et al.,* 1993 Vol 5 Tab 60]. Rather, the increased excretion of neutral sterols and possibly undigested dietary oil, could explain these effects [Kanauchi *et al.,* 1995 Vol 5 Tab 70].

Contrary to the studies with rats, significantly increased binding and excretion of bile acids was observed in other species. In rabbits fed a cholesterol-enriched diet containing chitosan, the excretion of bile acids was increased by 40% and cholic acid excretion was increased by 50%. The excretion of neutral sterols was also increased by 18% [Hirano *et al.,* 1995, as reported by Furda, 2000 Vol 4 Tab 40].

In broiler chickens, the bile acid concentration in the duodenum was reduced by 55% after feeding a diet containing 3% chitosan, indicating strong bile acid binding by chitosan [Razdan *et al.,* 1997 Vol 8 Tab 133]. In man, a diet containing 3 - 6 g/day of chitosan reduced serum cholesterol by 6.5% while increasing the excretion of total bile acids by 8-19%, when compared to the control period. After feeding, the excretion of bile acids increased due to the chitosan diet by as much as 62% [Maezaki *et al.,* 1993 Vol 6 Tab 98].

Based on these examples, in species like rabbits, chickens or men, the hypocholesterolemic effect of chitosan appears to achieved by binding and increasing the excretion of bile acids, as described by Furda's mechanistic Pathway 2B [Furda, 2000 Vol 4Tab 40].

5. Approaches to increase lipid and cholesterol lowering effects of chitosan

Ascorbic acid and sodium ascorbate

Fat digestibility in the presence of chitosan can be further reduced by adding ascorbic acid or sodium ascorbate to chitosan [Kanauchi *et al.*, 1994 and 1995 Vol 5 Tabs 69 and 70]. The synergistic effect of these two compounds with chitosan was demonstrated *in vivo*, specifically in rats, who were fed high fat diets containing 5% chitosan and either 1.5% ascorbic acid or sodium ascorbate. In a study which lasted two weeks, the amount of fecal lipids were, on average, 10% higher in the group consuming the diet containing chitosan plus ascorbic acid, compared with the group consuming chitosan alone. Analyses of the weight of the gastrointestinal contents, total fat digestibility, and the weight of the epididymal fat pads demonstrated a greater effect when the diet containing ascorbic acid or its sodium salt was fed.

The authors explained the synergistic effect of ascorbic acid with chitosan as acid-independent, and specific to ascorbic acid, which acts to reduce viscosity in the stomach, increase the oil holding capacity of the chitosan gel, and increase the flexibility of the chitosan-fat gel. The reduced gastric viscosity is believed to mediate effectively the emulsifying process of a lipid-soluble chitosan mixture in the stomach. Surprisingly, and as previously stated, in a separate experiment, the same authors found that the greater the *in vitro* viscosity of chitosan, the lower the fat digestibility in rats [Deuchi *et al.,* 1995 Vol 4 Tabs 28 and 29].

The key assumptions to this approach were that the dietary lipids are emulsified and engulfed in the stomach of the rats by chitosan, whose emulsification is enhanced by ascorbic acid or sodium ascorbate, and that the precipitation/solidification of a chitosan coat around the lipid droplets in the duodenum is immediate and impenetrable by pancreatic lipase. Therefore, there is no enzymatic hydrolysis of engulfed lipid droplets [Deuchi *et al.,* 1995 Vol 4 Tabs 28 and 29]. It is not surprising that sodium ascorbate has a similar effect to ascorbic acid, since it should be readily converted to ascorbic acid.

6. Demonstrations of the emulsification properties of chitosan in food models

The same principals, which are operable in the human body, with respect to the fat emulsifying properties of chitosan can also be utilized in food formulations. Particularly interesting from the point of view of a food scientist is the ability of chitosan to form emulsions with lipids in an acid environment, as discussed in the preceding sections. The following tables summarize the work of Stephen Poole, 1989, and Rodriguez, 2000, who researched the emulsification and foaming properties of chitosan in model food systems [Poole, 1988 Vol 11 Tab 225 and Rodriguez, *et al.,* 2000 Vol 12 Tab 237].

a) Behavior of Chitosan Emulsions and Droplet Formation

According to Rodriguez, *et al.,* 2000, chitosan appears to be a promising emulsifier, because it provides both electrostatic and viscosifying stabilization [Rodriguez, *et al.,* 2000 Vol 12 Tab 237]. Due to its properties as a polymeric surfactant, chitosan participates in the following mechanisms:

i. Chitosan, like polymeric surfactants, may attach to a surface via several segments, which gives it a large free energy of adsorption, despite a low free energy of adsorption per segment.

ii. It offers steric stabilization of dispersions unlike low molecular weight surface-active agents.

Together these mechanisms are referred to as electrostatic stabilization.

Rodriguez, *et al.*, 2000 determined the most efficient and economical chitosan/oil ratios to yield stable emulsions. Emulsions were prepared by adding a variable quantity of sunflower oil (20-120g) to a constant amount of 1% w/v chitosan in aqueous acetic acid (80g). Samples were stirred in a food processor for 3 minutes and a 65 ml aliquot was placed in a 100 ml cylinder. The elapsed time to phase separation was recorded. Three layers were observed when phase separation occurred: oil, white emulsion, and a dilute emulsion composed of very fine droplets. The researchers found that 86 g chitosan solution per 14 g oil and 80 g chitosan solution per 20 g oil (the second being the most economical) yielded the

most stable emulsions (Table 12) [Rodriguez, *et al.*, 2000 Vol 12 Tab 237].

Table 12
Behavior of Chitosan Emulsions[1]

Chitosan solution (1%)/ oil Proportion, g/g	Stability (1 h)
92/8	Two phases separation: Concentrated and dilute emulsion
86/14	No phase separation
80/20	No phase separation
70/30	Two phases separation: Concentrated and dilute emulsion
60/40	Three phases separation: Oil, concentrated and Dilute emulsion
40/60	Three phases separation: Oil, concentrated and Dilute emulsion

[1]Chitosan was sourced from shrimp.

b) Foam-enhancing Properties of Low-viscosity Chitosan

Small basic (cationic) proteins are so effective at enhancing the foaming properties of acidic proteins that they can tolerate the inclusion of lipids, which would otherwise prevent the foaming of solutions of acidic proteins alone. Chitosan is basic and interacts with proteins, but high molecular weight chitosan with a high viscosity does not foam, because the interaction with protein is so strong that a precipitate is formed [Poole, 1988 Vol 11 Tab 225].

Poole determined whether low viscosity chitosan behaves like basic proteins, which enhance the foaming of acidic proteins in the presence of lipids. Table 13 shows the effect of low-viscosity chitosan (1% in 1% acetic acid) on the foaming of a 6% whey protein isolate (WPI) system stressed with corn oil. The lipid prevented foaming of the WPI until chitosan was incorporated at a level of 0.6% or above. At that point, excellent foaming was observed [Poole, 1988 Vol 11 Tab 225].

Chitosan and the acidic protein were dissolved separately in 0.8% acetic acid. The oil or melted fat was emulsified with the protein solution and any carbohydrate added. The chitosan solution was then mixed with the protein solution and the pH was adjusted with 1M NaOH. The resulting mixture was whipped at full speed (200 rev/min) for 5 minutes in a Kenwood Chef Mixer. The prepared foam was then transferred to a graduated cylinder before the desired parameters were measured [Poole, 1988 Vol 11 Tab 225].

Table 13
Effect of Low-Viscosity Chitosan Concentration on the Foaming of Whey Protein Isolate
(WPI = 6.0%; corn oil = 10%; sucrose = 10%; pH = 6.0)

% Chitosan	%Foam Expansion	%Foam Volume Stability	%Foam Liquid Stability	Foam Description
0	120	60	15	Poor
0.1	140	65	15	Poor
0.2	100	60	20	Poor
0.4	100	60	20	Poor
0.6	550	100	90	Fairly Stiff
0.7	560	100	95	Very Stiff
0.8	560	100	95	Very Stiff

The lipid prevented foaming of WPI, but when chitosan was incorporated at a level of 0.6% (ratio = 1:10), foaming was excellent. As with basic protein, it is believed that this enhancing effect involves electrostatic interactions between the proteins molecules and chitosan at a pH value above the isoelectric point (pH 5.3) of the WPI [Poole, 1988 Vol 11 Tab 225].

Electrostatic interactions between acidic and basic species are by definition pH-dependent and foaming behavior reflects this, according to the author. Foam enhancement occurred within a fairly narrow pH range (5.5-6.5). Above pH 6.5 excessive precipitation prevented foaming.

Below pH 5.3, the protein was positively charged and did not interact strongly with chitosan. Hence careful control of pH would be required in processing operations. However, this

may be more than compensated by the benefits of being able to incorporate fat (e.g. cocoa or cocoa butter) in protein-based whipping formulations.

Table 14 shows that at a constant chitosan to WPI ratio of 1:10, the total concentration of whey protein and chitosan was also important. A minimum WPI concentration of 4% was required for foaming with 10% corn oil. At 8% excessive precipitation prevented foaming.

Table 14
Influence of WPI Concentration on Foaming with Corn Oil
(Chitosan: WPI = 1:10; corn oil = 10%; pH = 6.0; sucrose = 10%)

Conc. Of WPI (%)	Conc. Chitosan (%)	%Foam Expansion	%Foam Volume Stability	%Foam Liquid Stability	Foam Description
2.0	0.2	140	60	10	Poor
4.0	0.4	500	95	75	Fairly Stiff
6.0	0.6	550	100	90	Stiff
8.0	0.8	-	-	-	Dense Aggregate

The range of lipids used in food manufacture is extensive. The ability of the chitosan-whey protein system to tolerate several lipids was investigated (Table 15). Good foams were obtained with all the lipids tested with the exception of lecithin, a lipid that is known to be particularly detrimental to foaming [Poole, 1988 Vol 11 Tab 225]. Table 15 shows foaming scores on emulsions with and without low-viscosity chitosan added to whey protein isolate foaming systems. Chitosan significantly improved foam properties with all fats except lecithin.

Table 15
Foaming properties of WPI/chitosan with different lipids
(WPI = 6%; chitosan = 0.7%; sucrose = 30 %; pH = 6.0; lipid = 20%)

Type of fat/oil	Chitosan	%Foam Expansion	%Foam Volume Stability	%Foam Liquid Stability	Description of foam
Corn oil	+	570	100	100	Very stiff
	-	130	55	0	Poor
Coconut oil	+	870	100	100	Stiff
	-	80	45	0	Poor
Butter oil	+	910	100	75	Fairly Stiff
	-	130	50	0	Poor
Cocoa butter	+	630	100	100	Fairly Stiff
	-	95	45	0	Poor
Lard	+	300	85	35	Poor
	-	90	45	0	Poor
Lecithin (0.1%)	+	170	70	20	Poor
	-	200	65	0	Poor
Lard (10%)	+	970	90	100	Very Stiff
	-	90	45	0	Poor

Various sugars and polysaccharides are used in foods to sweeten or alter texture. A representative range was tested in a whey protein isolate foaming system. Sorbitol is a non-cariogenic sugar alcohol; glucose syrup consists of mono-, di-, and oligosaccharides derived by controlled hydrolysis of starch; polydextrose is a reduced-calorie bulking agent comprising randomly polymerized glucose; methylcellulose is an uncharged viscosity-enhancing food polysaccharide. Good foams were obtained when 0.7% of a chitosan solution was added to the foaming system, with all the saccharides except in the case of methyl cellulose, which inhibited foaming (presumably because the high viscosity inhibited air incorporation) [Poole, 1988 Vol 11 Tab 225].

Table 16 shows the effect of low-viscosity and hydrolyzed chitosans on the foaming of various proteins under similar conditions. Good foams were obtained with egg albumen, which, like whey protein, is a globular protein. However, hydrolysis of chitosan beyond 10% adversely effected foam-enhancement. Serum albumin (BSA), another acidic globular protein, also gave good results but no foam-enhancement was

observed with caseinate and gelatin, which are extended random coil molecules. Both were precipitated excessively by chitosan. Therefore, the conformation of the protein is an important factor to consider when adding chitosan to a foaming system.

Table 16
Effects of Low-Viscosity Chitosan and Hydrolyzed Chitosans
(DH 5, 10 and 15%) on the Foaming of Acidic Proteins
(protein = 6%; chitosan = 0.7%; sucrose = 30%; pH = 6.0)

Acidic protein	Chitosan	%Foam Expansion	%Foam Volume Stability	%Foam Liquid Stability	Description of foam
Egg albumen	LV	570	100	100	Very Stiff
Egg albumen	DH 5%	520	100	95	Moderately stiff
Egg albumen	DH 10%	500	95	75	Flowed
Egg albumen	DH 15%	235	80	35	Flowed
Caseinate	DH 5%	30	40	20	Very poor
Gelatin	DH 5%	-	-	-	No foam
WPI	DH 5%	720	100	100	Moderately stiff
BSA	DH 5%	710	95	75	Stiff

7. Conclusion

Chitosan has demonstrated an ability to improve foaming and emulsification systems in foods at relatively low percentages of use. Chitosan produced a w/o/w double emulsion in one step without the addition of surfactants [Rodriguez *et al.*, 2000 Vol 12 Tab 237]. These technical effects are based on the basic physicochemical properties of the molecule, which function *in vivo* as well as in food systems. Rodriguez *et al.*, 2000 hypothesizes that the one-step formation of w/o/w double emulsions may be due to the heterogeneity of the molecule [Rodriguez *et al.*, 2000 Vol 12 Tab 237]. The varied technical effects of which chitosan is capable will allow it to be considered as an ingredient in a wide variety of food categories.

L. Potential Human Toxicants

Chemical and heavy metal analyses of Primex chitosan products have shown that the products contain no toxicants of concern. Tables 5 through 9 above indicate that Primex chitosan contains less chemical and microbiological contaminants than many common food sources.

Contact Hypersensitivity of Primex Chitosan

Primex ASA commissioned a contact hypersensitivity test of Primex chitosan from RCC Ltd. in Füllinsdorf, Switzerland. The purpose of this skin sensitizing study was to assess the possible allergenic potential of Primex chitosan when administered topically to albino guinea pigs.

For this study the "Buhler Test" modified by Ritz, H.L. and Buhler, E.V. (1980) was used [Arcelin, 1999 Vol 3 Tab 8]. Twenty male albino guinea pigs (test group = 10) were treated topically with 7.5% of the Primex chitosan preparation once a week for a 3-week induction phase. Two weeks after the final induction application, the animals were challenged with the same test article preparation used for induction but at a concentration of 1.875%.

The ten animals of the control were not treated during the induction, but were treated once at challenge with 1.875 % of the Primex chitosan preparation.

The chitosan was prepared by dissolving one part of chitosan in bi-distilled water, warmed to 80º C, and vigorously homogenizing the solution with a magnetic stirrer. 0.6 parts of glycolic acid (70%) was added to the hot solution. The pH of the solution was adjusted to 5.5 with 1M NaOH as necessary. Bi-distilled water was added to fill the volume to 100 parts.

Selection of the concentration of the test article was based on the concentration of epidermal induction (the concentration that produced some irritation, but did not adversely affect the animals), and the epidermal challenge (the concentration that was the maximum tested non-irritant concentration) [Arcelin, 1999 Vol 3 Tab 8].

Results showed that none of the control and test animals were observed to have skin reactions after the challenge treatment with Primex chitosan at a concentration of 1.875 %.

M. Specifications for Food Grade Materials

1. Raw Materials

The following substances used in the manufacturing of Primex chitosan are food grade materials, or are used in accordance with current Good Manufacturing Practices (21 CFR 110.80(a)).

Sodium Hydroxide
Hydrochloric Acid
Hydrogen Peroxide
Chitin from Shrimp (*Pandalus borealis)*

2. Specifications of Raw Materials Set by Primex

Hydrogen Peroxide

Variables	Specifications
Hydrogen peroxide	20-60%
Stabilizer	<500 ppm

Section II Chemical Identity of Chitosan

Hydrochloric Acid

Variables	Specifications
Content	90-120% of the labeled value
Sulfate (as SO4)	≤ 0.48%(w/v)
Heavy Metals (as Pb)	≤ 10 μg/ml
Iron (as Fe)	≤ 30 μg/ml
Arsenic as (AS_2O_3)	≤ 2.0 μg/ml
Residue on Ignition	≤ 0.020%

Sodium Hydroxide

Variables	Specifications
Appearance	Colorless, mostly transparent
Content	90-120% of the labeled value
Sodium Chloride	≤ 2.0% per weight of NaOH
Heavy Metals (as Pb)	≤ 30 μg/g-NaOH
Mercury (as Hg)	≤ 0.10 μg/g-NaOH
Arsenic (as AS_2O_3)	≤ 4.0 μg/g-NaOH

Chitin

Variables	Specifications
Dry Matter	> 90%
Ash	< 2%
Flakes	0.1-6mm
Micromilled Powder	min. 100% < 60 mesh
Total Plate Count	< 1,000/g
Escherichia coli	negative
Coliform Bacteria	negative
Salmonella pr. 25gram	negative
Mould and Yeast	< 100/g

Notification Section III

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Section III
Information on Self-Limiting Levels of Use and Probable Consumption

A. Self-Limiting Levels of Use

While there are few published studies on the self-limiting levels of chitosan use in foods, several factors are expected to limit the use of the ingredient in food formulations. First, chitosan is a very expensive ingredient, with a value in excess of US $20 per pound. Second, studies by food scientists have shown that chitosan has been an effective emulsifier at percentages of use of less than 1.0% [Poole, 1988 Vol 11 Tab 225 and Rodriguez, *et al.,* 2000 Vol 12 Tab 237].

Excessive chitosan usage may produce aggregation of ingredients, depending on the synergies involved and the pH of the formulation [Poole, 1988 Vol 11 Tab 225]. Poole found that use of greater than 0.8% chitosan yielded a dense aggregate in a whey protein isolate-corn oil emulsion [Poole, 1988 Vol 11 Tab 225]. Further, Rodriguez *et al.,* 2000, stated that the affinity between chitosan and water decreased with increasing pH [Rodriguez, *et al.,* 2000 Vol 12 Tab 237].

Therefore, use of chitosan is expected to be limited by its physicochemical properties. Rodriguez *et al.,* 2000, concluded that the behavior of chitosan in systems containing different chitosan/oil ratios was due to certain properties of chitosan. The authors found that the surface tension of chitosan solutions (1% v/v acetic acid aqueous solution) was higher than that of the solvent (water). This implied that chitosan is strongly excluded from the air/solution interface. According to the researchers, the phenomena are consistent with expectations derived from the theoretical behaviors of the cationic $-NH_3^+$ and alcoholic –OH groups distributed on the molecules structure [Rodriguez *et al.,* 2000 Vol 12 Tab 237].

Primex submits that food processors will be involved in a great deal of applications development, in order to determine that most economical uses for chitosan.

B. Probable Consumption

A Realistic Estimate of Exposure to ChitoClear™ Chitosan

Primex ASA is providing a realistic estimate of exposure to

ChitoClear™ chitosan. The FDA's guidance document "*Estimating Exposure to Direct Food Additives and Chemical Contaminants in the Diet*" and USDA's *Continuing Survey of Food Intakes by Individuals* (CSFII, 1996) were used as references to estimate the realistic exposure of the chitosan produced by Primex ASA [Enns, *et al.,* 1997 Vol 4 Tab 36].

Realistic usage levels of chitosan were assigned to recognized food subcategories based on industry experience, applications trials, published articles, and economic considerations [MRCA, 1990 listed in Table 1]. The contribution for each major food category to chitosan exposure was calculated by adding the percentage usage levels in each major category, and then dividing by the number of food subcategories within a major category to obtain an average usage level for each major food category (See Table 1 below). The average usage levels were then multiplied by the average consumption for men and women as reported by the CSFII, 1996. This value was considered to be the mean level of intake. USDA's CSFII, 1996 was used to estimate mean intakes of the various food categories.

In order to calculate the 90th percentile of consumption, the mean intake level was multiplied by 2 as indicated the agency's guidance document. Additional exposure to chitosan may result from its use as a source of dietary fiber in dietary supplement form [Enns, *et al.,* 1997 and Langhelle, 1999 Vol 8 Tab 126]. Recommended dosages for chitosan as a fiber have source been determined by clinical trial [Colombo, *et al.*, 1996 Vol 3 Tab 24].

While the percentage of use assigned to each food subcategory was considered realistic, this exposure estimate remains conservative because 100% market penetration was assumed, and chitosan was assumed to be included in all products in a given subcategory. Additionally, the values presented below assume that 100% of consumers are "eaters" of chitosan- containing products within a given food category.

In reality, chitosan is expected to be used in only certain products within a given subcategory. Further, use within a subcategory will be limited by economics and organoleptic considerations. Market development in the US is likely to be directed towards the functional properties of chitosan, and certain functional properties may not be appropriate for a given consumer product. An example of this is that chitosan tends to increase viscosity in batter or fluid food systems. This may not create a desirable product in all cases.

Additionally, Primex ASA ChitoClear™ chitosan is a very expensive food ingredient, costing US$ 20 - 30 dollars per kilogram. Therefore, those applications in which it will be cost effective will need to be carefully chosen, and will most likely dictate that chitosan will be used at the minimum level necessary to produce a given technical or nutritional effect.

Table 1 below shows the realistic percentages of use assigned to each of the recognized food subcategories. Table 1 below shows the realistic percentages of use assigned to each of the forty-three food categories listed at 21 CFR § 170.3 (n). The correlation between the major food categories derived from the USDA food survey, and those listed at § 170.3 (n) is shown in Table 1.

The mean intake for selected major food subcategories is shown in Table 2 below. The sum of the grams of chitosan calculated from the mean intakes for each major food category listed on Table 2 is 7.97 grams per day. The agency's guidance document indicates that an estimate of the 90th percentile level of intake can be calculated by doubling the total mean intake. This value is 15.94 grams per day. Primex Ingredients ASA is also aware that certain individuals may consume chitosan as a dietary supplement. The recommended dose of chitosan as a dietary supplement is 1.5 to 3.0 grams per day. This dosage has been recommended based on the results of clinical trials with human volunteers [Colombo, *et al.*, 1996, Vol 3 Tab 24].

The mean and the 90th percentile of estimated exposure to Primex ChitoClear™ chitosan remains lower than the recommended levels of intake for dietary fiber (25-30 grams per day) in the United States [Enns, *et al.,* 1997 Vol 4 Tab 36]. Additionally, dietary fiber intake in the US averaged for men and women has been estimated by USDA at 16.1 grams per day [Enns, *et al.,* 1997 Vol 4 Tab 36]. This value still represents a deficit in relation to levels established for good health. It is possible that consumption of chitosan may provide health benefits from additional dietary fiber intake. Further, no untoward effects have been reported in multiple studies involving chitosan consumption by human volunteers consuming similar amounts of chitosan [See Section IV, Safety]. Therefore, Primex ASA submits that the exposure to chitosan represented by this estimate will pose no threat to the public health.

Section III Information on Self-Limiting Levels of Use and Probable Consumption

Table 1
Use of ChitoClear™ Chitosan In the Food Categories Listed by the US FDA At 21 CFR §170.3 (n)

	Food Categories	Realistic Percentage of Use in the US Market*
01	Baked Goods, Baking Mixes	1.28%
02	Beverages alcoholic	0%
03	Beverages and beverage bases, non-alcoholic	0.625%
04	Breakfast Cereals	1.6%
05	Cheeses	0.56%
06	Chewing Gum	0%
07	Coffee and tea**	0%
08	Condiments and relishes	0.143%
09	Confections and frostings	0
10	Dairy product analogs	0%
11	Egg Products	0.213%
12	Fats and oils	0.22%
13	Fish Products	0.72%
14	Fresh eggs	0.213%
15	Fresh fish	1.5%
16	Fresh fruits and fruit juices	0.46%
17	Fresh Meats	0.5%
18	Fresh poultry	0.5%
19	Fresh vegetables	0.46%
20	Frozen dairy desserts and mixes	0.31%
21	Fruit and water ices	0
22	Gelatins, puddings and fillings	0%
23	Grain products and pastas	1.28%
24	Gravies and Sauces	0%
25	Hard candy	0
26	Herbs, seeds, spices, seasonings, blends	0
27	Jams and jellies, home prepared	0
28	Jams and jellies commercial	0.25%
29	Meat products	0.14%
30	Milk, whole and skim	0
31	Milk products	0.77%
32	Nuts and Nut products	0%
33	Plant protein products	3.06%
34	Poultry products	0.055%
35	Processed fruits and fruit juices	0.46%
36	Processed vegetables and vegetable juices	0.21%
37	Snack foods	0.75%
38	Soft Candy	0.5
39	Soups, home prepared	0
40	Soups and soup mixes	0.85%
41	Sugar, white granulated	0.25%
42	Sugar substitutes	0%
43	Sweet Sauces, toppings, and syrups	0%

Section III Information on Self-Limiting Levels of Use and Probable Consumption

Table 2
Estimated Intakes of ChitoClear™ Chitosan
From Selected Food Categories

Food Category	Use level (%)	Food Intake (g)	CH Intake (g)	CSFII
Baked goods, baking mixes				
Yeast Breads and Rolls	1.28	50	0.64	1996
Quick breads, pancakes, etc.	2.0	20	0.4	1996
Cakes, cookies, pastries, pies*	2.0	38	0.76	1996
Mixtures mainly grain	2.0	107	2.14	1996
Breakfast cereals				
Ready-to-eat cereals	1.6	17	0.272	1996
Grain products and pastas				
Rice	1.28	19	0.243	1996
Pasta	1.28	21	0.269	1996
Snack foods (crackers, chips)	0.75	12	0.09	1996
Total Vegetables**		132		1996
Fresh vegetables	0.21	66	0.139	1996
Processed vegetables, juices	0.21	66	0.139	1996
Total Fruits		162		1996
Citrus juices	0.46	59	0.271	1996
Dried fruits	0	1	0	1996
Non-citrus juices and nectars	0	26	0.120	1996
Fruits and mixtures	0	18	0	1996
Milk products				
Yogurt	0.77	8	0.066	1996
Milk desserts	0.77	24	0.185	1996
Cheese	0.56	16	0.089	1996
Milk-Based Beverages†	2.0	34	0.68	1966

*Includes Dietetic Bars
**No Potatoes

Section III Information on Self-Limiting Levels of Use and Probable Consumption

Table 2 (continued)

Meat Products				
Sausages, processed meats	0.14	21	0.029	1996
Fresh Meat	0.5	34	0.17	1996
Poultry	0.5	20	0.10	1996
Fish	1.11	11	0.122	1996
Eggs				
Egg Products	0.213	18	0.038	1996
Legumes				
Legumes	2.2	28	0.616	1996
Nuts and Nut Products				
Nuts and Nut Products	0	3	0	1996
Fats and Oils				
Salad dressings	0.22	8	0.176	1996
Total sugars and sweets				
Candy (Soft and Hard)	0.5	7	0.35	1996
Other Sugar Products‡	0.25	15	.0375	1996
Beverages Non-Alcoholic				
Coffee	0	254	0	1996
Teas	0	128	0	1996
Low Cal. Fruit drinks and -ades	1.0	18	0.18	1996
Low Cal. Carbonated drinks	0	74	0	1996
Total Intake (grams)			**7.97**	
Dietary Supplement	1.5		**1.5 - 3.0**	
Total Intake with supplement use			**9.47 -10.97**	

†Skim Milk
‡Jams, Jellies, etc.

Section III Information on Self-Limiting Levels of Use and Probable Consumption

Table 3
USDA Major Food Categories And Corresponding Categories From 21 CFR 170.3 (n)

USDA Food Category[1]	Categories Listed In 170.3 (n)[2]
Total Grain Products	01, 04, 23, 37
Dietary Supplement; Source of Soluble Fiber	
Vegetables and Vegetable Dishes	36
Fruits and Fruit Preparations	16, 35
Milk Desserts/Cheese Products	5, 20, 22, 31,10
Meat, Poultry and Fish	29, 34.
Total Fats and Oils	12
Legumes and Legume Dishes	33, 36
Sugars and Sweets	06, 09, 25, 28, 38, 42, 43
Nuts and Seeds	32
Total Non-Alcoholic Beverages (excluding coffee and fruit juices)	16, 35, 03

1. Major food categories are those used in USDA's Continuing Survey of Food Intake by Individuals [Enns, *et al.,* 1997 Vol 4 Tab 36].
2. Column 2 contains the number assigned to the various food categories as listed in 21 CFR § 170.3 (n), which are contained within those reported by Enns, *et al.*, 1997 [Enns, *et al.,* 1997 Vol 4 Tab 36].

Notification Section IV

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Section IV
Detailed Summary of the Basis for the Determination That Primex Chitosan is GRAS

A. Introduction

A balanced review of the published literature and unpublished information contained in this document concerning chitosan supports the conclusion that it is a safe food ingredient for human consumption. Various animal and human studies have shown that chitosan is well tolerated, and that its metabolism produces substances that are found in the human body and other dietary substances [Kelly, 1998 Vol 12 Tab 226].

In addition to providing information on the basic chemistry and metabolism of chitosan, this report reviews several pre-clinical and clinical studies that have been preformed to examine the safety of chitosan ingestion in various species including man. Ten human studies published in peer-reviewed journals are discussed below, including several with placebo controlled double blind designs. Results of these studies are summarized in Table 6.

A number of published toxicity assessments on chitosan have been conducted within the context of biomedical applications. Where appropriate, data from those studies has been included in separate sections to support the conclusion that chitosan does no harm to body tissues or processes. The totality of the published animal and human studies on chitosan suggest that there are no significant or consistent untoward effects attributable to its consumption, even when the substance is consumed at levels equivalent to several percent of the diet.

Both chitosan, and its raw material, chitin are biocompatible and are found indigenously in the cell walls of various fungi, crustaceans and insects [Illum, 1999 Vol 5 Tab 61]. Chemically chitosan, a cationic polysaccharide, is the N-deacetylated product of chitin. The structure of chitosan is mainly that of a polymer of D-glucosamine and N-acetyl-D-glucosamine [Aspden, *et al.,* 1977 Vol 3 Tab 10]. Both chitin and chitosan are considered to be nitrogenous polysaccharides. The structure of the chitin molecule is similar to that of cellulose, but it is composed of units of 2-acetylamino-2-deoxy-D-glucopyranose, while chitosan is composed of ß (1->4)-2-amino-2-deoxy—D-glucopyranose and -acetylamino-2-deoxy-D-glucopyranose bound [1 --> 4] by glycosidic bonds [Chobot, *et al.,* 1995 Vol 3 Tab 22]. These

polysaccharides occur particularly in the fungal kingdom, in invertebrate animals, and to a small degree in higher plants [Chobot, *et al.,* 1995 Vol 3 Tab 22].

Chitosan can also be chemically derived by deacetylating its raw material, chitin. Commercially, chitin is obtained from the cuticles of sea animals, which are waste products from food industry. The technologies for obtaining chitin from lower fungi [e.g. the genus Aspergillus] have been elucidated [Chobot, *et al.,* 1995 Vol 3 Tab 22], but they are not considered economically viable.

The term chitosan refers to a family of polymers whose members differ in their degree of deacetylation (i.e., ratio of N-acetylated to N-deacetylated subunits) and molecular weight. These two characteristics are fundamental to the physicochemical properties of the chitosans and therefore have considerable influence on their biological activities [Aspden, *et al.,* 1997 Vol 3 Tab 10].

Because of the high cost of producing chitosan, it has not been historically used as a food or food ingredient. However, modern humans, continue to consume small quantities of chitosan as part of a normal diet. Natural sources of chitosan in the diet include various fungi and animals [Illum, 1998 Vol 5 Tab 61]. Both chitin and chitosan are inherent to a large extent in food fungi, e.g. *Pleurotus ostreatus* [Chobot, *et al.,* 1995 Vol 3 Tab 22]. Knowledge of ancient diets suggests that the amount of chitosan consumed by our ancestors was much greater than at present. However, the presence in the human intestinal system of certain enzymes that specifically cleave chitosan into its component chemical units (chitinase) strongly suggests that humans have the capacity to safely consume chitosan at much greater concentrations than are common in a Western diet [Muzzarelli, 1997, Vol 7 Tab 104]. The fact that the human body has evolved physiological processes to metabolize chitosan, the results from several recent animal toxicology studies, and a history of commercial consumption of chitosan in Japan, convincingly support the position that the consumption of chitosan as a macronutrient would pose no risk to the consumer.

Over the last decade, coinciding with the commercial production of higher purity chitosans, much interest and experimental activity has centered on the use of such chitosans in a great diversity of novel food and biomedical applications. Chitosans have been found to have many beneficial effects, including immunostimulation, cholesterol reduction, anticoagulant (e.g. sulfated derivatives) and hemostatic activities, and

wound-healing and anti-microbial properties [Aspden, *et al.,* 1997 Vol 3 Tab 10]. All of these properties have been found to be based on the natural chemistry and structure of the material.

Several authors have cited that great attention has been paid to chitin and chitosan in recent years. Chobot, *et al.,* 1995 discussed the great interest in the antihypercholesterolemic and antihyperlipidemic activities, of chitosan, both of which have been experimentally demonstrated. In practice certain medicinal preparations and functional food products with protective activity against certain risk factors of atherosclerosis have already been commercialized [Chobot, *et al.,* 1995 Vol 3 Tab 22]. Additionally, the anticoagulative and anti-aggregative effects of sulfated derivatives of chitosan have been demonstrated. The research in this field is, however, in its early stages. Both polysaccharides (chitin and chitosan) have been introduced into pharmaceutical technology where they have been cited as very effective drug vehicles. Promising results have been achieved in experiments with antineoplastics [5-fluorouracil and methotrexate], with nonsteroidal antiphlogistics [ibuprofen] and others. Several authors publishing in this area have considered it very advantageous that chitin and chitosan possess low toxicity, little allergenic potential, and exert moderate immunostimulating effects [Chobot, *et al.,* 1995 Vol 3 Tab 22]. The mechanism by which chitosan interacts with the body to produce these effects has been discussed in Section II.

B. Data Relied on to Establish Safety

While only Primex Chitosan has been characterized in detail in this document, all information presented relates to the safety of chitosan as a family of substances. The following pages review data and information relative to the safety and efficacy of chitosan as a food ingredient.

In establishing the safety of Primex chitosan, the company relied on peer reviewed published material; company sponsored studies, and decades of human experience with chitosan consumption in Japan, China, and other Pacific Rim nations.

C. Exposure

The mean and the 90th percentile of estimated exposure to Primex ChitoClear™ chitosan (7.97 and 15.94 grams respectively) remains lower than the recommended levels of intake for dietary fiber (25-30 grams per day) in the United States [Enns, *et al.,* 1997 Vol 4 Tab 36]. Additionally, dietary fiber intake in the US averaged for men and women has been estimated by USDA at 16.1 grams per day [Enns, *et al.,* 1997 Vol 4 Tab 36]. This value still represents a deficit in relation to levels established for good health.

Primex Ingredients ASA is aware that certain individuals may consume chitosan as a dietary supplement. The recommended dose of chitosan as a dietary supplement is 1.5 to 3.0 grams per day. This dosage has been recommended based on the results of clinical trials with human volunteers [Colombo, *et al.*, 1996, Vol 3 Tab 24]. It is possible that consumption of chitosan may provide health benefits from additional dietary fiber intake. Further, no untoward effects have been reported in multiple studies involving chitosan consumption by human volunteers consuming similar amounts of chitosan [See Section IV, Safety]. Therefore, Primex ASA submits that the exposure to chitosan represented by the estimate provided in Section III above, will pose no threat to the public health.

In reality, exposure to Primex chitosan is expected to be limited by:

1. Cost - only certain products within a given food subcategory will be of high enough value to support an ingredient costing US$ 20 - 30 dollars per kilogram.
2. Organoleptic considerations – use of chitosan may not provide the desired organoleptic targets for all
3. Functional properties - certain functional properties may not be appropriate for a given consumer product.

D. Technical Effects

Applications for chitosan include general use in foods as a multiple-use direct additive. The ingredient should be used under the conditions of current Good Manufacturing Practice.

In order to classify the various effects ingredients may have in food, FDA has published a list of 32 physical or technical functional effects for which direct food ingredients may be added to food. These are codified at 21 CFR §170.3 (o) (1-32). Technical effects for chitosan are

covered under several of the following terms as listed under 21 CFR §170.3 (o).

(3) "Antioxidants": Substances used to preserve food by retarding deterioration, rancidity, or discoloration due to oxidation.
(5) "Curing and pickling agents": Substances imparting a unique flavor and/or color to a food, usually producing an increase in shelf life stability.
(6) "Dough strengtheners": Substances used to modify starch and gluten, thereby producing a more stable dough, including
the applicable effects listed by the National Academy of Sciences/National Research Council under "dough conditioner."
(8) "Emulsifiers and emulsifier salts": Substances which modify surface tension in the component phase of an emulsion to establish a uniform dispersion or emulsion.
(14) "Formulation aides": Substances used to promote or produce a desired physical state or texture in food, including carriers, binders, fillers, plasticizers, film-formers, and tableting aids, etc.
(16) "Humectants": Hygroscopic substances included in food to promote retention of moisture, including moisture-retention agents and antidusting agents.
(20) "Nutrient supplements": Substances which are necessary for the body's nutritional and metabolic processes.
(24) "Processing aids": Substances used as manufacturing aids to enhance the appeal or utility of a food or food component, including clarifying agents, clouding agents, catalysts, flocculents, filter aids, and crystallization inhibitors, etc.
(28) "Stabilizers and thickeners": Substances used to produce viscous solutions or dispersions, to impart body, improve consistency, or stabilize emulsions, including suspending and
bodying agents, setting agents, jellying agents, and bulking agents, etc.
(29) "Surface-active agents": substances used to modify surface properties of liquid food components for a variety of effects, other than emulsifiers, but including solubilizing agents, dispersants, detergents, wetting agents, rehydration enhancers, whipping agents, foaming agents, and defoaming agents, etc.
(31) "Synergists": Substances used to act or react with another food ingredient to produce a total effect different or greater than the sum of the effects produced by the individual ingredients.
(32)"Texturizers": Substances which affect the appearance or feel of the food.

E. Contaminants and Manufacturing

Product Purity

Primex Ingredients has performed a number of tests on its products to assure the purity of the product. Analyses for contaminants are shown in Table 1 below. The analysis demonstrated that the product was free of the products of most concern [Primex Ingredients, Contaminant Data, 2000 Vol 7 Tab 123]. Likewise, Table 2 below demonstrates that Primex chitosan is much lower in dioxins than other common foodstuffs, and lower than other raw materials from which chitosan is made (crabs) [Primex Ingredients Dioxin Testing, 2000 Vol 7 Tab 120]. Table 3 below demonstrates that the shrimp used as raw material in the Primex process are free of the White Spotted Virus and the Yellow Head Virus (WSSV and YHV) known to infect shrimp [University of Arizona Virus Scan, 2000 Vol 3 Tab 1].

Section IV Detailed Summary of the Basis for the Determination that Primex Chitosan is GRAS

Table 1
Contaminant Analysis
(Translated from the original Norwegian by the Laboratory)

NORWEGIAN INSTITUTE FOR WATER RESEARCH P.O. Box 173 Kjelsaas, 0411 OSLO	TEST REPORT
Name	PRIMEX
Contract number	99019-01
Date of sample reception	25.01.99
Laboratory Code	137 1-2
Sample Type	Biological matter (chitosan)
Concentrations in	Micrograms/kg wet weight (pp., w.w.)
Analytical method	H2-4
Date of analysis	12.03.99
Analyst	Lasse Berglind
1: Primex Chitosan batch no. 355 2: Primex Chitosan batch no. 355	

Parameter/sample no.	**1**	**2**
Naphthalene	<0,5	<0,5
2-Methyl-naphthalene	<0,5	<0,5
1-Methyl-naphthalene	<0,5	0.9
Biphenyl	2.7	4.6
2,6-Dimethylnaphthalene	<0,5	<0,5
Acenaphthalene	<0,5	<0,5
Acenaphthene	0.7	1.3
2,3,5-Trimethylnaphthalene	<0,5	<0,5
Fluorene	1.6	1.3
Phenanthrene	3.9	2.9
Anthracene	<0,5	<0,5
1-Methylphenanthrene	1.1	<0,5
Fluoranthene	3.1	3.6
Pyrene	1.9	2.7
Benzo(a)anthracene*	<0,5	0.8
Chrysene/triphenylene	0.9	0.9
Benzo(b,j,k)fluoranthene*	1.2	1.2
Benzo(e)pyrene	<0,5	<0,5
Benzo(a)pyrene*	<0,5	<0,5
Perylene	<0,5	<0,5
Indeno (1,2,3-cd) pyrene*	<0,5	<0,5
Dibenzo (a, c/a, h) anthracene* 1)	<0,5	<0,5
Benzo (ghi) perylene	0.6	0.5
Total PAH	17.7	20.7
Sum carcinogenic PAH*	1.2	2
% Carcinogenic PAH	6.8	9.7
% Dry substance	91.9	92.2

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Table 1 (continued)

*Potentially carcinogenic according to Int. Agency for Research on Cancer (1987)
1) Only the dibenzo (a, h)-isomer

This test report may only be copied completely and without changes.
The test result is only valid for the sample, which has been tested.

Table 2
Dioxin Analysis

Sample	Num -ber	Concentration (pg TE/g ww)[1] Average	Concentration (pg TE/g ww)[1] min-max	Fat (%)	Concentration (pg TE/g fw)[2]
Cod Fillet	2	0.1	0.03-0.2	0.3	33
Mackerel	2	1.5	0.7-2.2	16	4.4-14
Herring	6	2.3	1.8-2.8	14	16
Farmed Salmon	4	0.7	0.5-1.1	3	23
Cod Liver	2	7.7	6.8-8.5	58	13
Cod liver Oil	4	5.8	0.6-16	100	5.8
Crab	3	29	17-44	20	145
Margarine	4	1	0.9-1.1	81	1.2
Butter	3	1.2	0.9-1.4	81	1.5
Lambs fat	3	1.3	0.5-2.6	80	1.6
Egg	3	0.2	0.1-0.2	11	1.8
Milk	8				0.3-0.9
Milk (near industries)	5				1.3-3.1
Primex Ingredients for PCDD/PCDF and non-ortho PCB[3]	1	0.07			

[1] TE =2378-TCDD toxicity equivalents according to the international model (NATO/CCMS, 1989); pg TE/g ww = a measure of the toxicity equivalents per wet weight.
[2] pg TE/g fw = a measure of the toxicity equivalents per fat weight
[3] Measured as polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans in pg i – TE/g

Table 3
Virus Analysis

UAZ #	Sample ID	WSSV	YHV
00-131A/1	Shrimp shells	Not detected	Not detected
00-131A/2	Shrimp shells	Not detected	Not detected
00-131B/1	ChitoClear: TM661	Not detected	Not detected
00-131B/2	ChitoClear: TM761	Not detected	Not detected
00-131B/3	ChitoClear: TM93	Not detected	Not detected

F. Safety Studies

a) Chitosan and Cholesterol

In recent years, chitosan has been introduced as a dietary supplement with cholesterol lowering activity. The mechanism behind this apparent activity has been linked to the ability of chitosan to effect the lipid transport mechanism in the gut. During lipid metabolism, free fatty acids (released from consumed fat), cholesterol, bile salts, and other components from mixed micelles are released in the small intestine. This process comprises an essential step in the fat absorption process. It has been hypothesized, that the positively charged chitosan can bind to free fatty acids and bile salt components, and hence disrupt micelle/lipid absorption [Illum, 1998 Vol 5 Tab 61].

At very low concentrations *in vitro* chitosan acts as a very efficient "emulsion breaker". It causes the fusion of small micelles of fat (with negative charges on the micelle surface) into larger micelles, which then have a lower surface to volume ratio. Eventually chitosan causes separation of the fat and oil into two separate phases [Furda, 2000 Vol 4 Tab 30].

Several investigators have published studies in which animal models were used to study the effect of dietary chitosan on cholesterol levels. Those studies are summarized below in Table 4. It is important to note that chitosan was fed at levels up to 7.5% of the diet in these studies without toxicological effect.

Table 4
Hypocholesteremic and Hypolipidemic Effects of Chitosan in Animals

Species	Dosage of Chitosan	Duration	Effect	Reference
Rats	5% of diet	13 days	42% reduction (corn oil) 24% reduction (lard)	Deuchi, *et al.*, 1995a
Rats	5% of diet	13 days	74% reduction Apparent Fat digestibility 84.5% reduction in Apparent Fat digestibility with sodium ascorbate	Deuchi, *et al.*, 1995b
Rats	5% of diet	1 test meal	49.6% reduction in free phospholipids in intestine	Ebihara, *et al.*, 1989
Lymph Cannulated Rats Fed intragastrically	8% of a fat emulsion	1 emulsified Meal	45.7% reduction in cholesterol uptake with safflower oil	Ikeda *et al.*, 1989
Rats	2-5% of the diet	20 days	25-30% reduction in plasma cholesterol	Sugano *et al.*, 1980
Rats	5% of the diet	21 days	73.5-83.2% reduction in Liver Lipids	Sugano *et al.*, 1988
Rats	5% of the diet with 1% cholesterol and 0.2% Cholic Acid	21 days	50% reduction in Liver Lipids;69.5% reduction in Liver cholesterol (in comparison to cellulose)	Jennings *et al.*, 1988
Rats	5-7.5% of the Diet, with 1% Cholesterol and 0.2% Cholic Acid	1, 3, or 6 weeks	54% Reduction in Plasma Cholesterol and 64% reduction in Liver Cholesterol with 5% dietary Chitosan; 7.5% Dietary Chitosan Maintained Cholesterol Homeostasis	LeHoux, *et al.*, 1993

Section IV Detailed Summary of the Basis for the Determination that Primex Chitosan is GRAS

Table 4 Continued

Chickens	30g/kg	21 days	26% reduction in Ileal Fat Digestibility	Razdan *et al.*, 1994
Chickens	30g/kg	13 Days	Reduced body weights and feed intakes; increase in plasma HDL/cholesterol: total cholesterol ratio;	Razdan *et al.*, 1997

b) Animal Safety Studies

Table 5 below summarizes animal studies, in which the toxicological effects of chitosan have been analyzed. Several of the studies mentioned desirable attributes of chitosan, when delivered as part of a diet.

Muzzarelli, 1997 stated that chitosan is a non-toxic natural polysaccharide of β-1,4-linked glucosamine residues that has been produced industrially by deacetylation of chitin obtained from crab and prawn shells [Muzzarelli, 1997 Vol 7 Tab 104]. Because of chitosan's well-recognized physiological adaptability, it is used as a food additive and in biomedicinal materials, etc. Also, because chitosan is not hydrolyzed by the digestive enzymes of man, and its chemical structure is similar to that of cellulose, it can be used as a new source of dietary fiber. However, because chitosan contains one amino group per residue, the author felt that its cationic character might confer properties quite different from those of other dietary fibers, and that most of its chemical functions were attributable to the amino group. Among them, the most noticeable dietary consideration is its adsorption of acidic metabolites such as uric acid and bile acid [Jing, *et al.*, 1997 Vol 5 Tab 67]. Its hypocholesterolemic effect on healthy man has also been reported [Sugano *et al.*, 1988 Vol 9 Tab 156, Maezaki *et al.*, 1993 Vol 6 Tab 98, and Jing, *et al.*, 1997 Vol 5 Tab 67].

Table 5
Toxicity Assessments of Chitosan in Animals

Species	Dosage of Chitosan	Duration	Effect	Reference
Mice	5 mg subcutaneous injection; Oral administration of 5% chitosan in a casein-based diet	Every two weeks for 12 weeks; Oral administration for 12 weeks	Injections did not evoke general cellular abnormalities; With oral administration, mouse body weights decreased, and probiotic bacterial populations decreased	Tanaka, *et al.*, 1997
Mice	1 mCi/kg body weight of ^{166}Ho-Chit (DW-166 HC)	Single Dose	Slightly decreased kidney weights, without histological alterations	Lee *et al.*, 1998
Sprague-Dawley Rats	5% of diet	21 days	No Mucosal change in large bowel; No untoward effects; Chitosan did not alter hemoglobin concentration, and showed least change in serum iron (-1.8%) among oat gum, cholestyramine, and Chitosan	Jennings, *et al.*., 1988
Rats	5-7.5% of the Diet, with 1% Cholesterol and 0.2% Cholic Acid	1, 3, or 6 weeks	No change in liver weight or appearance	LeHoux, *et al.*, 1993
One-Day-Old Broiler-Chicks	30 g/kg	21 Days	Reduced Short Chain Fatty Acids shows Antimicrobial Nature of Chitosan	Razdan, *et al.*, 1994
One-Day-Old Broiler-Chicks	30 g/kg	21 Days	Chitosan reduced body weights and Feed Intakes	Raman, *et al.*, 1997

Section IV Detailed Summary of the Basis for the Determination that Primex Chitosan is GRAS

Table 5 (Continued)

Rats	0.5, 2, 5 and 10 % of Diet	81 Days	No Constipation or diarrhea; 0.5% increased HDL in cholesterol-free diets; 2% & 5% did not influence food intake or growth; Finer particles at 2% restrained growth	Sugano, *et al.*, 1980
Oral Toxicity in Rats and Mice	Chitosan	Being Translated	LD_{50} was reported to be 16g/kg	Illum, 1998, reporting on Arai, *et al.*, 1968
Rats	5% Diet		Urinary Excretion of Calcium Increased	Wada, *et al.*, 1997
Mice	Chitosan Microspheres	60 Days	Minimized drug toxicity	Jameela, *et al.*, 1996
Mice	300 to 1,500 mg/kg	8 Days	Prevented the side effects and toxicity of a cancer-inhibiting drug.	Kimura, *et al.*, 1999
Mice Rabbits Guinea Pigs	72 hours Eye Irritation Tests Skin Irritation Tests	Canadian Grant Needed	No Undesirable Toxic Effects	Rao, *et al.*, 1997
Rabbits		Canadian Grant	No irritability to Keratinocytes	Augustin, *et al.*, 1995
Sprague-Dawley Rats	5% of Diet with High Fat	2 Weeks	Decrease in Mineral Absorption and Bone Mineral Content; Additional Calcium Required; Decrease in serum Vitamin E Levels	Deuchi, *et al.*, 1995

G. Human Safety

Overweight and obesity is a prevalent and costly threat to public health. Compelling evidence links overweight and obesity with serious disorders such as cardiovascular diseases and diabetes. However, dietary regimens are notoriously burdened with poor compliance. Chitosan is promoted in the US and other countries as an oral remedy to reduce fat absorption and has now been incorporated as a major constituent into several dietary supplements. For these reasons, several studies have recently been published, which have investigated the effect of chitosan on weight and cholesterol reduction in humans.

A number of recent human studies have examined the effect of daily doses of 1.0 to 6.0 grams of chitosan, when taken as a dietary supplement. These studies have measured important physiological parameters related to vitamin and mineral balance. No significant untoward effects were noted in over 310 individuals tested, even when chitosan was fed for periods of up to four weeks.

Table 6 below summarizes the effects of chitosan on both healthy overweight individuals, and those with compromised health conditions. Interestingly, a group of human studies conducted in Italy utilized the same source of chitosan as a supplement to the diet. The studies differed in their caloric restrictions, from no dietary restriction to a 1000 calorie/day diet. In those studies, volunteers consumed from 1.0 to 1.6 grams of chitosan per day (see below). Results of the several studies showed that chitosan intake did not alter mineral or hematological balances in the human subjects as compared to the controls. The authors also concluded that chitosan was more effective at contributing to weight loss, when accompanied by a low-calorie diet.

The effects of chitosan were investigated in eighty patients with renal failure undergoing long-term hemodialysis treatment. The patients were tested after a control treatment period of 1 week. Half were fed 30 chitosan tablets [45 mg chitosan/ tablet] three times a day (a total of 1.350 g/day). Ingestion of chitosan effectively reduced total serum cholesterol levels [from 10. 14 ± 4.40 to 5.82 ± 2.19 mM] and increased serum hemoglobin levels [from 58.2 ± 12.1 to 68 ± 9.0 g/L]. Significant reductions in urea and creatinine levels in serum were observed after 4 weeks of chitosan ingestion. The feeling of physical strength, the appetite and the sleep of patients in the treatment group had improved significantly after 12 weeks of ingestion, compared with those of patients in the control group. During the treatment period, no clinically problematic symptoms were observed. These data suggest

that chitosan might be effective in reducing hypercholesteremia for renal failure patients, although the authors stated that the mechanism of the effect should be investigated further [JING, *et al.,* 1997 Vol 5 Tab 67].

Section IV Detailed Summary of the Basis for the Determination that Primex Chitosan is GRAS

Table 6
Effect of Chitosan on Human Subjects

Subjects	Dosage of Chitosan	Duration	Effect	Reference
40 Humans with Renal Failure	10 (45 mg) Tablets 3 X per day	12 weeks	Reduced Serum cholesterol 42.6% and Increased Serum Hemoglobin Levels 16.8%; No Problematic Symptoms Were Observed	Jing. *et al.*, 1997
Human Males; 8 Volunteers; Ages 20-23	3-6 g/day	2 weeks	Decrease in serum cholesterol Of 6.4% And 9.8% increase in HDL	Maezaki, *et al.*, 1993
15 Over weight Human Volunteers; Ages 18-60	1.0 Gram/day with Unrestricted Diet	28 Days	No change in BMI, serum cholesterol, triglycerides, Vitamins A, D, E, or β carotene. Vitamin K was significantly increased. No serious adverse effects were reported	Pittler *et al.*, 1999
51 Healthy Obese Women	1.2 grams/day of Microcrystalline Chitosan with no change in eating habits	8 Weeks	Chitosan was well-tolerated and no serious adverse events or changes in safety parameters, including fat-soluble vitamins A and E, and serum iron and transferrin were noted.	Wuolijoki, *et al.*, 1999
20 Obese Subjects; Ages 30-80	4 Chitosan Tablets per day (1.0 g twice daily) With and Without Dietary Restriction; Low calorie diet was 1200 calories	One Month	Tolerance was good, no side effects or abnormal blood chemistry were reported; Some constipation reported; administration of laxatives was rarely required	Macchi, 1996

Section IV Detailed Summary of the Basis for the Determination that Primex Chitosan is GRAS

Table 6 (Continued)

50 Obese Patients; Ages 26-65	4 Tablets per day of Chitosan With Low (1100) Calorie Diet	4 Weeks	Adverse Effects Mild and transient; Not significantly different from controls; 4.2% with Chitosan, 6.4% with placebo	Giustina, *et al.*, 1995
45 Obese Subjects; Ages 25-70; Mean 46.7 ± 13.2	4 Tablets per day of Chitosan (1600 mg) in 2 doses; With Low Calorie Diet	4 Weeks	Mild and Transient Effects in 2.3% of Chitosan Subjects; Not Statistically Different from the Control	Sciutto, *et al.*, 1995
40 Subjects; Ages 32-70; Mean 52.4 ± 16.7	4 Tablets; 1600 mg Chitosan divided into 2 doses; With 1000 cal. diet	4 Weeks	Adverse effects (2.6%) were mild and transient nausea; Compared to constipation (5.4%) with placebo; No pathological or clinical change in blood chemistry or hematological data	Veneroni, 1996
7 Healthy Men; Arterial Blood	5 grams of Chitosan Powder	Single Dose	No Adverse Effects Reported; Chitosan Prevented Blood Pressure increase following a high-salt meal	Kato, *et al.*, 1994
43 Obese Subjects; Ages 30-69; Mean 48.3 ± 12.5	4 Tablets per day (1600 mg) in 2 doses; of Chitosan With Low Calorie Diet	4 Weeks	Mild and Transient Adverse Effects in 5% of the Chitosan Subjects (flatulence); Not statistically different from the controls.	Colombo, *et al.*, 1995

More recently, the primary aim of a study by Pittler, *et al.,* 1999 was to investigate the clinical effectiveness of oral chitosan for body weight reduction. Thirty-four overweight volunteers were included in a randomized placebo-controlled double-blind trial. Subjects were assigned to receive either four capsules of chitosan or indistinguishable placebo twice daily for 28 consecutive days. Measurements were taken at baseline, and after 14, and 28 days of treatment. Subjects maintained their normal diet and documented the type and amount of food consumed. Adverse effects were assessed and compliance was monitored. Data from 30 subjects were entered into an intention-to-treat analysis. After four weeks of treatment, body mass index, serum cholesterol, triglycerides, vitamins A, D, E and β-carotene were not significantly different in subjects receiving chitosan compared to those receiving placebo. Vitamin K was significantly increased after four weeks in the chitosan group compared with the placebo group [$P<0.05$]. Compliance was 91.5% and 96.0% for the chitosan and placebo groups, respectively. In conclusion, the data suggested that chitosan in the administered dosage, but without dietary alterations, did not reduce body weight in overweight subjects. No serious adverse effects were reported [Pittler, *et al.,* 1999 Vol 7 Tab 117].

Peroral microcrystalline chitosan [MCCh; 3 capsules, each 400 mg b.i.d.] or placebo was given for 8 weeks in a double-blind manner to 51 healthy obese women just before routine hospital and home meals. Weight records, serum lipids [total, LDL and HDL cholesterol, triglycerides] and safety laboratory parameters were monitored before the trial and at 4, 6 and 8 weeks of treatment. In a subgroup of subjects with a body mass index > or = 30, who had not changed their eating habits, serum LDL cholesterol decreased 0.57 ± 0.72 mmol/l [n = 11] at 4 weeks in the MCCh group and 0.10 ± 0.60 mmol/l [n = 14] in the placebo group [$p < 0.05$]. At 8 weeks, LDL cholesterol reduction was 0.48 ± 0.91 mmol/l in the MCCh group and 0.26 ± 0.57 mmol/l in the placebo group [$p > 0.1$]. In all subjects, the reduction in LDL cholesterol at 4 weeks was 0.48 +/- 0.72 mmol/l [n = 24], in MCCh subjects, and 0.18 ± 0.58 mmol/l [n = 27] in placebo subjects [p = 0.057], and 0.52 ± 0.69 mmol/l and 0.31 ± 0.63 mmol/l, respectively, at 8 weeks [$p > 0.1$]. MCCh did not significantly alter serum total and HDL cholesterol [$p > 0.1$], but slightly increased serum triglycerides compared to placebo [p = 0.015- 0.06]. No reductions in weight were observed in any treatment group. Chitosan was well tolerated and no serious adverse events or changes in safety laboratory parameters were noted including serum fat-soluble vitamins A and E, and serum Fe++ and transferrin [Wuolijoki, *et al.,* 1999 Vol 11 Tab 221].

A very important trial was conducted to determine the weight loss and the lipid-lowering effects obtained with a new chitosan dietary fiber (a mixture of chitosan, guar's meal, ascorbic acid and other micronutrients) and a caloric restriction in a weight-reducing program for 90 obese subjects [Colombo, *et al.*, 1996 Vol 3 Tab 24]. In the randomized, double blind, placebo controlled trial, subjects were treated with a hypocaloric diet plus 4 tablets/day of chitosan dietary fiber or with a hypocaloric diet plus 4 tablets/day of placebo for 4 weeks. The chitosan content of each tablet was 400 mg.

The subjects in the study discussed above consisted of 90 adult patients (age range from 25 to 70 years; mean 46.7 + 13.2 years) of both sexes (45 men and 45 women). Inclusion criteria included ambulatory subjects aged between 20 an 70 years, mild obesity (overweight between 10% and 25% as regards the normality weight-height tables), mild hypertension, and hyperlipoproteinemia. Exclusion criteria included patients with known or presumed hypersensitivity to any component of the supplement (active substances and excipients); those who did not guarantee total adherence to the trial protocol; and patients with severe hepatic or gastrointestinal diseases, renal insufficiency, severe chronic disease, pregnancy, or concomitant administration of drugs that could interfere with the results of the study. If these drugs were taken previously, they were stopped 30 days before the new treatment schedule.

The patients were randomized (random number tables) into two groups containing the same number of subjects [Colombo, *et al.*, 1996 Vol 3 Tab 24]. The groups were normalized for sex composition, mean age, body weight, body height, and percentage of overweight, systolic and diastolic pressures, arterial pressure, serum total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides. No significant differences using Chi-square and Student's t-test appeared between the treatment groups [Colombo, *et al.*, 1996 Vol 3 Tab 24].

The study showed a statistically significant reduction in body weight, overweight, arterial systolic and diastolic pressure, total and LDL cholesterol, and triglycerides and an augmentation of HDL cholesterol in both the groups. However, with the diet, which included chitosan, the variations were statistically greater than in the placebo group.

Body weight reduction was 7.19 kg in the chitosan group and 3.36 kg in the placebo group, overweight reduction was 1.45% in the chitosan group and 3.8% in the placebo group. Arterial systolic pressure

reduction was 12.1 mm Hg in the chitosan group and 2.8 mm Hg in the placebo group.
Arterial diastolic pressure reduction was 12.4 mm Hg in the chitosan group and 3.8 mm Hg in the placebo group. Total cholesterol reduction was 25.3% in the chitosan group and 11.1% in the placebo group. LDL cholesterol reduction was 32.1% in the chitosan group and 13.6% in the placebo group. HDL cholesterol augmentation was 11.8% in the chitosan group and 4.6% in the placebo group.

Mild and transient side effects were reported in 2.3% of the chitosan treated subjects (nausea) and in 6.8% of the subjects in the placebo group (nausea or constipation), without any statistically significant difference between the two groups. No pathologic or clinically significant changes in blood chemistry or hematological assay were observed.

The authors concluded that the data from this study suggested that diet plus a chitosan dietary fiber was a very useful treatment for overweight in obese subjects, and that their weight loss was associated with an evident reduction in hypertension and hyperlipoproteinemia [Colombo, *et al.*, 1996 Vol 3 Tab 24]. Significantly, subjects consuming chitosan maintained normal mineral and hematological parameters throughout the trial (See Tables 7 and 8 below).

Table 7
Effect of Chitosan on Mineral Balance in Humans

Treatment	Parameter	Normal Val.	Before	After	Statistics*
Diet + Chitosan	Sodium (meq/L)	137-145	140.16±15.21	140.14±15.18	N.S.**
	Potassium (meq/L)	2.7-3.9	3.12±0.29	3.13±0.30	N.S.**
	Calcium (meq/L)	2.1-3.0	2.66±0.32	2.61±0.31	N.S.**
	Magnesium (meq/L)	2.0-2.5	2.31±0.27	2.30±0.28	N.S.**
	Zinc (Hg/dL)	75-120	94.06±9.11	92.78±9.51	N.S.**
	Iron (Hg/dL)	80-180	101.68±11.67	100.44±10.62	N.S.**
	Copper (Hg/dL)	70-140	106.11±9.41	102.57±10.55	N.S.**
	Vitamin A (Hg/dL)	20-100	55.32±6.83	51.40±7.02	N.S.**
	Vitamin D (nmol/L)	20-100	63.59±7.21	60.64±7.02	N.S.**
	Vitamin E ((Hg/dL)	1.5-4	2.67±0.31	2.53±0.42	N.S.**
Diet + Placebo	Sodium (meq/L)	137-145	142.33±15.75	141.62±16.37	N.S.**
	Potassium (meq/L)	2.7-3.9	3.19±0.28	3.18±0.29	N.S.**
	Calcium (meq/L)	2.1-3.0	2.64±0.34	2.63±0.33	N.S.**
	Magnesium (meq/L)	2.0-2.5	2.32±0.29	2.31±0.29	N.S.**
	Zinc (Hg/dL)	75-120	93.15±10.06	91.22±9.22	N.S.**
	Iron (Hg/dL)	80-180	102.78±11.29	100.03±11.96	N.S.**
	Copper (Hg/dL)	70-140	113.52±10.96	111.029.45	N.S.**
	Vitamin A (Hg/dL)	20-100	58.21±6.76	56.326.31	N.S.**
	Vitamin D (nmol/L)	20-100	62.67±6.93	64.287.61	N.S.**
	Vitamin E ((Hg/dL)	1.5-4	2.74±0.36	2.700.39	N.S.**

*Comparison before-after with Student's t-test
** N.S. = difference not significant

Section IV Detailed Summary of the Basis for the Determination that Primex Chitosan is GRAS

Table 8
Effect of Chitosan on Hematological Parameters

Treatment	Parameter	Normal val.	Before	After	Statistics *
Diet + Chitosan	Hemoglobin (g/dL)	12-18	14.02±1.33	14.14±1.82	N.S.**
	Hematocrit (%)	38-54	42.86±5.16	42.36±5.66	N.S.**
	Leukocytes (no.)	4500-11000	8930±1920	9210±2150	N.S.**
	Erythrocytes (millions/ml)	4.2-6.2	4.66±0.51	4.69±0.61	N.S.**
	Glucose (mg/dL)	70-110	97.4±93.52	95.22±9.64	N.S.**
	Urea nitrogen (mg/dL)	8-23	20.11±2.94	19.23±2.87	N.S.**
	Creatine (mg/dL)	0.6-1.2	0.92±0.33	0.95±0.31	N.S.**
	Bilirubin (mg/dL)	0.1-1.2	0.58±0.36	0.56±0.30	N.S.**
	AST (U/L)	5-30	22.06±3.28	24.39±3.46	N.S.**
	ALT (U/L)	5-35	26.61±3.07	25.44±3.19	N.S.**
	Gamma-GT (U/L)	5-50	21.17±2.54	20.72±2.77	
Diet + Placebo	Hemoglobin (g/dL)	12-18	14.21±1.92	14.17±1.78	N.S.**
	Hematocrit (%)	38-54	42.81±6.02	43.02±5.56	N.S.**
	Leukocytes (no.)	4500-11000	7790±2420	8760±2210	N.S.**
	Erythrocytes (millions/ml)	4.2-6.2	4.45±0.50	4.52±0.55	N.S.**
	Glucose (mg/dL)	70-110	94.08±10.12	96.71±9.66	N.S.**
	Urea nitrogen (mg/dL)	8-23	19.65±2.71	19.74±2.59	N.S.**
	Creatine (mg/dL)	0.6-1.2	0.91±0.36	0.93±0.32	N.S.**
	Bilirubin (mg/dL)	0.1-1.2	0.55±0.24	0.57±0.28	N.S.**
	AST (U/L)	5-30	26.323.77	23.11±3.86	N.S.**
	ALT (U/L)	5-35	28.343.06	26.78±3.11	N.S.**
	Gamma-GT (U/L)	5-50	22.422.79	21.56±2.82	N.S.**

*Comparison before-after with student's t-test.
** N.S. = difference not significant.

000195

The effects of the introduction of the same chitosan dietary fiber preparation were tested over a four-week weight-reducing regimen for the treatment of obesity in a randomized, double blind, placebo controlled trial performed with 100 obese subjects. Group 1 (50 patients) was treated with a low caloric diet plus 4 tablets/day of placebo. Group 2 (50 patients) was treated with a low caloric diet plus 4 tablets/day of the chitosan preparation. Each tablet contained 400 mg of chitosan [Guistina, *et al.,* 1995 Vol 4 Tab 43].

The subjects in this trial were informed verbally and in writing about their rights, the aims and possible risks of the study, and each gave written consent. 100 adult patients (age range from 26 to 65 years; mean 43.9 =12.1 years) of both sexes (54 men and 46 women) were admitted to the trial.

The groups were normalized for sex composition, mean age, body weight, body height, percentage of overweight, systolic and diastolic pressures, arterial pressure, and heart and respiratory rate. No significant differences were calculated, using Chi-square and Student's t-test, between the treatment groups [Guistina, *et al.,* 1995 Vol 4 Tab 43]. The dose was two tablets twice a day, at the main meals (lunch and dinner) for four weeks. The weight-reducing regimen was a low caloric diet of about 1000-1100 kcal based on 34% fats, 41% carbohydrates and 25% proteins [Guistina, *et al.,* 1995 Vol 4 Tab 43].

At the end of the study Guistina observed a statistically significant reduction in body weight, overweight, arterial, systolic, and diastolic pressures, and respiratory rate in both the groups. In the chitosan treated subjects, the reductions were statistically greater than in placebo group Body weight decreased from 83.6 Kg to 76.3 Kg in the chitosan group and from 82.3 to 79.3 kg in the placebo group. Overweight decreased from 17.2% to 7.3% in the chitosan group, and from 16.3% to 12.4% in the placebo group. Arterial systolic pressure decreased from 145.3 mm Hg to 135.8 mm Hg in the chitosan group and from 146 to 142.9 in the placebo group. Arterial systolic pressure dropped from 92.6 mm Hg in the chitosan group to 84.2 mm Hg, and from 92.5 to 90.0 in the placebo group. Respiratory rate dropped from 27.6/min to 21.2/min in the chitosan group, and from 28.1/min to 26.3/min in the placebo group. [Guistina, *et al.,* 1995 Vol 4 Tab 43].

Adverse effects (always mild and transient) were reported in very few patients (4.2% in the chitosan treated group and 6.4% in the placebo group, without any statistically significant difference between the two groups). On the basis of what the authors considered to be a good

level of statistical confidence in the clinical evidence, diet plus a chitosan dietary fiber was found to be very useful for the treatment of obesity and the secondary disorders of hypertension dyspnea. The authors concluded that chitosan in addition to a low caloric diet was more effective than a low caloric diet alone [Guistina, *et al.,* 1995 Vol 4 Tab 43].

Inclusion criteria involved ambulatory subjects with ages between 20 and 70 years, mildly obese subjects (overweight between10% to 25% in comparison to normality weigh-height tables), and subjects with mild hypertension in the study. Exclusion criteria were eliminated patients with known or presumed hypersensitivity to any component of the supplement (active substances or excipients); subjects who did not guarantee a total adherence to the trial protocol; and those with severe hepatic or gastrointestinal diseases, renal insufficiency, severe chronic disease, pregnancy, and concomitant administration of drugs that could interfere with the results of the study. If these drugs were taken previously they were discontinued 30 days before the new treatment schedule. The subjects were randomized (random number tables) into two groups containing the same number of subjects [Guistina, *et al.,* 1995 Vol 4 Tab 43].

Macchi, 1996, indicated that it was common knowledge that diabetes increases the risk of premature death, hyperlipidemia, arterial blood hypertension and ischemic heart diseases. Both for medical and aesthetic considerations, the need for safe and natural methods to improve the weight reducing process was obvious in particular if the substance enhanced the loss of body fat with a saving of muscular mass [Macchi, 1996 Vol 6 Tab 96]. Macchi stated that a new chitosan dietary fiber (CT), which was indigestible, and had a very low toxicity profile had been demonstrated to have antihyperlipidemic effects. The fiber appeared to interfere with fat absorption. After ingestion CT was solubilized in the acid environment of the stomach, where it entrapped fatty acids by means of ionic bonding, and neutral fats by means of hydrophobic forces, thereby decreasing their intestinal absorption [Macchi, 1996 Vol 6 Tab 96].

Macchi, 1996 conducted a study, whose purpose to evaluate the effects of CT associated with a low calorie diet or with a habitual free diet, compared to placebo (P), on body weight (BW), body mass index (BMI), body fat (BF), skinfolds (S), arterial blood pressure (BP) and blood chemistry analysis. Thirty obese subjects were studied in a randomized double blind controlled trial for a period of one month. Group A was treated with 4 tablets/day of CT plus a 1200 calorie diet;

group B with the same hypocaloric diet and 4 tablets/day of placebo; group C with 4tablets /day of CT without dietary restriction. No side effects or abnormal modifications of blood chemistry analysis were noted at the end of the study. Subjects taking CT appeared satisfied with the treatment, but some complained of constipation. The study showed a statistically significant ($p<0.001$) reduction of BW, BMI, BF, S in all the groups, but it was greatest in group A (BW= - 4Kg; BMI= -1/7 Kg/cm; BF= - 4%; S= -17 mm in group A: BW= -2.6Kg; BMI = -0.6 Kg/cm; BF= -1.2%; S= -5 mm in group B; BW = -2.8 Kg; BMI= -1.1 Kg/cm; BF = -3.3%; S = -11 mm in group C).

The investigators considered it worth emphasizing, that even if BW reduction was similar in groups B and C, subjects treated with CT (group C) showed a statistically significantly greater skinfold decrease. It can be argued that subjects treated with CT (alone or in association with a hypocaloric diet) experienced a greater loss of adipose tissue in comparison to subjects only on diet restriction [Macchi, 1996 Vol 6 Tab 96].

Diastolic blood pressure, significantly decreased in groups A (-7 mm Hg) and C (-9 mm Hg) ($p<0,001$), but not in group B (-1 mm Hg n.s.). The authors hypothesized that the greater loss in BF may be responsible for the effect [Macchi, 1996 Vol 6 Tab 96].

Plasma cholesterol and triglyceride levels decreased significantly ($p<0.01$) in all groups (-26 mg/dl and - 27mg/dl in group A; -15 mg/dl and -26 mg/dl in group B; -26mg/dl and-27mg/dl in group C), but there was a significant increase in HDL-cholesterol (=11mg/dl, $p <0,001$) only in group C.

In conclusion Macchi's study demonstrated that the dietary fiber CT was useful in the treatment of obesity, because it enhanced the loss of body fat and acted to normalize plasma cholesterol and triglycerides levels, perhaps with a concomitant increase in HDL-cholesterol. Greater effects were obtained when CT was associated with a hypocaloric diet [Macchi, 1996 Vol 6 Tab 96].

In another randomized, double-blind, placebo-controlled trial body weight loss and lipid-lowering effects obtained with a caloric restriction and the same dietary fiber (a mixture of chitosan, guar's meal, ascorbic acid and others micronutrients) as used by diet Macchi, 1996 and Colombo, 1996 were investigated in a weight-reducing program for 80 obese adult subjects with hyperlipidemia [Veneroni, *et al.,* 1996 Vol 11 Tab 214]. The subjects were treated with a hypocaloric diet (1000

calories) plus 4 tablets/ day of chitosan dietary fiber (1.6 g) or with a hypocaloric diet plus 4 tablets/ day of a placebo for 4 weeks. At the end of the study period a statistically significant reduction in body weight and overweight, triglycerides and total LDL cholesterol, and an augmentation of HDL cholesterol were observed in both groups but in the chitosan treated group the differences were substantially greater than in placebo group [Veneroni, *et al.,* 1996 Vol 11 Tab 214].

Body weight reduction was 7.9 Kg in the chitosan group and 3.4 Kg in the placebo group, overweight reduction was 11.2% in the chitosan group and 4.2% placebo group, total cholesterol reduction was 23.9% in the chitosan group, and 10.4% in the placebo group, LDL cholesterol reduction was 33.4% in the chitosan group and 12.1% in the placebo group, triglyceride reduction was 23.5% in the chitosan group and 9.3% in the placebo group. HDL augmentation was 10.2% in the chitosan group and 3.5% in the placebo group.

Adverse events were reported in 2.6% of the chitosan treated subjects (mild and transitory nausea) and in 5.4% of the subjects in the placebo group (constipation) without any statistically significant difference between the two groups [Veneroni, *et al.,* 1996 Vol 11 Tab 214].

No pathologic or clinically significant changes in blood chemistry or hematological data were observed. From the results obtained in the study, the diet plus a chitosan dietary fiber appeared to be a useful treatment (more effective than diet alone) of the overweight and hyperlipidemia obese subjects [Veneroni, *et al.,* 1996 Vol 11 Tab 214].

80 adult patients (age ranging from 32-70 years mean; 52.4 ± 16.7 years) of both sexes (37 men and 43 women) were admitted to the trial. Inclusion criteria included subjects who filled the following criteria: ambulatory, age between 20 and 70 years, mildly obese (overweight between 10% to 25% as compared to normality weight-height tables) and hyperlipidemic. Exclusion criteria included subjects with known or presumed hypersensitivity to any component of the supplement (active substances or excipients), subjects who did not guarantee total adherence to the trial protocol, severe hepatic or gastrointestinal diseases, renal insufficiency, severe chronic disease, pregnancy, concomitant administration of drugs that could interfere with the results of the study. If these drugs were taken previously they were stopped 30 days before the new treatment schedule [Veneroni, *et al.,* 1996 Vol 11 Tab 214].

A trial was conducted to evaluate the efficiency and tolerability of the same chitosan dietary fiber tested by Veneroni, 1995 and Macchi, 1996 (a mixture of chitosan, Guar's meal, ascorbic acid and others micronutrients) in association with a low caloric diet in a weight-reducing program for 86 obese subjects. In the randomized, double blind, placebo controlled trial, subjects were treated with the hypocaloric diet plus 4 tablet/day of chitosan dietary fiber (1.6 g/d) or with a hypocaloric diet plus 4 tablets/day of placebo for 4 weeks [Sciutto, *et al.,* 1995 Vol 8 Tab 141].

At the end of the study a statistically significant reduction in body weight, overweight, total and LDL cholesterol, and triglycerides was observed in both groups. An increase in HDL cholesterol in the group treated with diet + chitosan was also observed, but in diet + chitosan group the variations were statistically greater than in diet + placebo group.

In order to verify the possible interference of chitosan with intestinal absorption of mineral salts or fat-soluble vitamins the authors determined blood concentrations of sodium, potassium, calcium, magnesium, zinc, iron, copper, and vitamins A, D, and E.

No significant changes were noted in blood concentrations of these substances after the treatment and there was no difference between the two treatment groups [Sciutto, *et al.,* 1995 Vol 8 Tab 141].

Mild and transient adverse effects were reported in 5% of the chitosan-treated subjects (flatulence) and in 18.4% of subjects in the placebo group (nausea and/or constipation), without any statistical differences between the two groups [Sciutto, *et al.,* 1995 Vol 8 Tab 141].

The authors' findings suggest that diet plus the new chitosan dietary fiber is a very useful treatment of the overweight in obese subjects and that the weight loss is associated with an evident reduction in hyperlipidemia without any significant interference of intestinal absorption of mineral salts or fat-soluble vitamins (See Table 9 below) [Sciutto, *et al.,* 1995 Vol 8 Tab 141].

The studies discussed in the section above have indicated that human subjects are able to tolerate chitosan at the levels given, even when engaging in a restricted diet, without adverse effects. It is significant to note that subjects receiving placebo experienced mild and transient effects from the low calorie diets alone.

Section IV Detailed Summary of the Basis for the Determination that Primex Chitosan is GRAS

Table 9
Effect of Chitosan on Hematological Parameters

Treatment	Parameter	Before	After	Statistics*
	Hemoglobin (g/dL)	14.28±1.46	14.34±2.06	N.S.**
	Hematocrit (g)	43.38±5.64	43.51±6.07	N.S.**
	Leukocytes (no.)	7840±1866	7756±1904	N.S.**
	Erythrocytes (millions/ml)	4.51±0.53	4.62±0.59	N.S.**
	Glucose (mg/dL)	92.57±10.37	91.65±9.02	N.S.**
	Urea Nitrogen (mg/dL)	19.78±2.76	19.67±2.50	N.S.**
	Creatinine (mg/dL)	0.89±0.25	0.90±0.29	N.S.**
	Bilirubin (mg/dL)	0.56±0.32	0.510.29	N.S.**
Diet +	AST (U/L)	28.41±3.55	25.443.11	N.S.**
Chitosan	ALT (U/L0	25.09±3.88	23.193.26	N.S.**
	Gamma-GT (U/L)	19.04±2.72	18.56±2.59	N.S.**
	Sodium (mEq/L)	137.7±13.0	138.9±13.8	N.S.**
	Potassium (mEq/L)	4.55±0.24	4.54±0.21	N.S.**
	Calcium (mEq/dL)	9.07±0.81	9.09±0.79	N.S.**
	Magnesium (mEq/L)	1.92±0.20	1.91±0.25	N.S.**
	Ferritin (ng/ml)	106.4±12.71	105.1±14.7	N.S.**
	Iron(ng/dL)	109.0±11.9	110.2±12.3	N.S.**

Section IV Detailed Summary of the Basis for the Determination that Primex Chitosan is GRAS

Table 9 (continued)

Diet + PLACEBO	Hemoglobin (g/dL)	13.91±2.16	14.07±1.94	N.S.**
	Hematocrit (g)	43.78±5.11	43.66±5.01	N.S.**
	Leukocytes (no.)	6905±2086	7125±1945	N.S.**
	Erythrocytes(millions/ml)	4.38±0.41	4.49±0.48	N.S.**
	Glucose (mg/dL)	96.12±9.27	94.33±9.82	N.S.**
	Urea Nitrogen (mg/dL)	19.43±2.66	19.28±3.09	N.S.**
	Creatinine (mg/dL)	0.90±0.30	0.88±0.29	N.S.**
	Bilirubin (mg/dL)	0.53±0.19	0.54±0.20	N.S.**
	AST (U/L)	27.84±3.57	26.78±3.14	N.S.**
	ALT (U/L0	22.41±2.93	21.49±2.78	N.S.**
	Gamma-GT (U/L)	19.38±2.23	19.11±2.15	N.S.**
	Sodium (mEq/L)	140.2±13.1	139.1±12.6	N.S.**
	Potassium (mEq/L)	4.50±0.21	4.53±0.26	N.S.**
	Calcium (mg/dL)	9.05±0.77	9.08±0.73	N.S.**
	Magnesium (mEg/L)	1.98±0.22	1.96±0.23	N.S.**
	Ferritin (ng/ml)	104.1±13.3	105.7±12.4	N.S.**
	Iron (ng/dL)	105.2±12.0	107.9±11.8	N.S.**

*Comparison before-after with Student's t-test.

**N.S. = difference not significant

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1. Fate of Chitosan in the Gastrointestinal Tract

Chitosan has been characterized as a polymer of glucosamine, which meets most of the criteria of dietary fibers with hypocholesterolemic potential [Fukada, *et al.,* 1991 Vol 4 Tab 38 and Sugano, *et al.,* 1988 Vol 9 Tab 156]. These criteria include nondigestibility in the upper gastrointestinal tract, high viscosity, a polymeric nature, and high water-binding capacity, which becomes reduced in the lower gastrointestinal tract. Sugano, *et al.,* 1988, stated that chitosan might not be hazardous to the morphology and functioning of the intestine because of its fiber-like behavior and its low toxicity [Sugano, *et al.,* 1988 Vol 9 Tab 156].

The metabolic fate of chitosan in the digestive tract of man has been tracked through radiolabelled preparations [Macleod, *et al.,* 1999 Vol 6 Tab 97]. A study was carried out to assess the potential of pectin:chitosan:hydroxypropyl methylcellulose [HPMC] [P:C:H] films for colonic drug delivery. Radiolabelled [^{99m}Tc] tablets were coated with a 3:1:1. P:C:H film and administered to four healthy male human volunteers. The gastro-intestinal transit of the tablets was assessed by gamma scintigraphy. The results showed that in all cases, the tablets were able to pass through the stomach and small intestine intact. Degradation of the tablets began once they had reached the colon, due to digestion of the coat by colonic bacteria. The study highlighted the potential of this coating system for colonic drug delivery, and demonstrated that chitosan is not degraded until it reaches the colon [Macleod, *et al.,* 1999 Vol 6 Tab 97].

The amount of radioactivity released in relation to tablet position was measured in one healthy male volunteer. Results showed that all the radioactivity remained (105.7%) through the small intestine (Table 10 below). However, only 38.0% of the radioactivity remained at the end of the ascending colon at 335 minutes post ingestion. After 405 minutes in the digestive tract, only 11.3% remained in the transverse colon [Macleod, *et al.,* 1999 Vol 6 Tab 97].

Table 10
Tablet Position and Radioactivity Released with Time After Administration to One Volunteer

Time after tablet administration (min)	Estimated radioactivity remaining (%) in tablet	Estimated tablet position
2	100.0	Stomach
15	101.2	Stomach
30	102.7	Stomach
60	105.0	Small intestine
120	103.3	Small intestine
180	105.7	Small intestine
260	90.7	Ileo caecal junction
275	66.4	Ascending colon
290	63.5	Ascending colon
305	49.0	Ascending colon
335	38.0	Ascending colon
365	16.7	Spread into transverse colon
405	11.3	Spread into transverse colon

The hydrolysis of chitosan has been studied, and the degradation of natural polymers, such as chitosan and chitin proceeds via enzymatic degradation [Domard, *et al.*, 1989 Vol 12 Tab 227 and Lee *et al.*, 1995 Vol 6 Tab 91]. The mechanism of enzymatic attack on a polymer is usually specific with regard to the coupling of the enzyme to the polymer, and the site of the initial degradation. Both chitin and chitosan have been known to be digestible by chitinase and lysosyme in accordance with the quantity of N-acetyl groups and their distribution within the backbone of the molecule [Lee, *et al.*, 1995 Vol 6 Tab 91, Aiba, 1993 Vol 12 Tab 228, Aiba, 1992 Vol 12 Tab 229, and Sashiwa, *et al.*, 1990 Vol 12 Tab 230].

Muzzarelli, 1997, has provided evidence for the degradation of chitosan in humans via non-specific chitinase and lysosymal enzyme systems [Muzzarelli, 1997 Vol 7 Tab 104]. The highest lysozyme susceptibility was shown by a degree of deacetylation of approximately 70% [Sashiwa, *et al.*, 1990 Vol 12 Tab 230]. The authors indicated that chitosans with degrees of deacetylation of approximately 70% have also been reported to have the highest immunoadjuvant activity through mouse peritoneal macrophage activation [Sashiwa, *et al.*, 1990 Vol 12 Tab 230]. Other authors have demonstrated the existence of bacterial chitosanases in nature [Gooday, 1990 Vol 4 Tab 25].

Chitosan is degraded into chitooligosaccharides, and then finally into glucosamine [Domard, 1989, Vol 12 Tab 227, Aiba, 1993 Vol 12 Tab 228, and Aiba, 1992 Vol 12 Tab 229]. Enzymatic methods have been developed for determining the degree of deacetylation of chitosan [Nanjo, *et al.*, 1991, Vol 12 Tab 231].

Glucosamine, which is the final breakdown product of chitosan is also formed in the human body as glucosamine 6-phosphate (G6-P) [Kelly, 1998 Vol 11 Tab 226]. It is the most fundamental building block required for the biosynthesis of certain classes of compounds, such as glycolipids, glycoproteins, glycosaminoglycans (formerly called mucopolysaccharides), hyaluronate and proteoglycans, which require amino sugars. Because it is a component of all these compounds, it is an essential component of cell membranes and cell surface proteins as well as interstitial structural molecules that hold cells together. Kelly, 1998 states that directly or indirectly, glucosamine plays a role in the formation of articular surfaces, tendons, ligaments, synovial fluid, skin, bone, nails, heart valves, blood vessels, and mucus secretions of the digestive, respiratory, and urinary tracts [Kelly, 1998 Vol 11 Tab 226].

Glucosamine (2-amino-2-deoxy- alpha-D-glucose) is one of the two hexosamine sugars (6 carbon amino sugars) common in animal cells (the other being galactosamine). Structurally, glucosamine is modified glucose with an NH3 group replacing the OH group found on carbon two (C-2). G6-P is an aminomonosaccharide (amino sugar) produced in the body by the combination of glutamine with fructose, through the enzymatic action of glucosamine synthetase [Kelly, 1998 Vol 11 Tab 226].

Glucosamine is found in many tissues and secretions in the body, and is the primary amino sugar substrate for the biosynthesis of macromolecules, such as chondroitin sulfate and hyaluronic acid, which provide the framework for collagen formation [Kelly, 1998 Vol 11 Tab 226].

The synthesis of G6-P begins with the structural rearrangement of glucose 6-phosphate to fructose 6-phosphate to facilitate interaction with the amino acid glutamine. The enzyme glucosamine synthetase facilitates the transfer of an amide group (NH3) from glutamine to fructose 6-phosphate. The enzyme simultaneously isomerizes (creates an intramolecular rearrangement of a compound without any net change of the components of the compound) this compound to form G6-P. The resulting G6-P molecule is the precursor to all hexosamines and hexosamine derivatives. This first biotransformation of glutamine and fructose 6-phosphate to G6-P is considered the rate-limiting step in amino sugar biosynthesis, and is an essential step in the glycosylation of all proteins [Kelly, 1998 Vol 11 Tab 226].

Setnikar, *et al.*,1984 studied the fate of glucosamine when taken orally [Setnikar *et al.*,1984 Vol 12 Tab 232]. Using a rat model, blood levels, tissue distribution and excretion patterns of radioactivity were studied in after administration of [^{14}C] glucosamine sulphate by either the intravenous or oral route. After intravenous administration, plasma radioactivity declined in the first 30 min, then increased, reaching a peak at the 2nd hour, and disappeared, with a half-life of 28 hours. The radioactivity was reported to have diffused rapidly in the tissues. The liver and kidney levels were found to be higher than those in plasma. There was early incorporation of radioactivity in the skeletal tissues (cartilage and bone). About 50% of the administered radioactivity was excreted with expired CO_2 and about 35% was excreted in the urine. Fecal excretion was small (2% of the administered dose). After oral administration, radioactivity was quickly found in plasma, where it reached a peak at 4 hours. Plasma levels then declined slowly, with biphasic kinetics. The tissue distribution, including uptake in the

skeleton, were similar to those seen with intravenous administration. Fecal excretion was minor, demonstrating that glucosamine was nearly completely bioavailable when administered orally. The large excretion (82%) with expired CO_2 indicated that glucosamine is to a large extent broken down to smaller fragments [Setnikar *et al.*,1984 Vol 12 Tab 232].

Two later studies by Setnikar, *et al.,* (1986 and 1993) investigated the metabolism of glucosamine or glucosamine sulphate in dogs and man. The pharmacokinetics, organ distribution, metabolism and excretion of glucosamine were studied in the dog by administering uniformly labeled [^{14}C]-glucosamine (sulfate), either i.v. or orally, in single doses. Immediately after i.v. administration, the radioactivity in plasma resulting from the labeled glucosamine, freely diffused into organs and tissues. The investigators found that the radioactivity disappeared quickly from plasma with an initial half life of 13 minutes, and a terminal half life of 118 min. After 30-60 min the radioactivity in the plasma was no longer due to glucosamine, but was incorporated into alpha-and beta globulins. The protein-incorporated radioactivity was detected within 20-30 min after i.v. administration. The signal reached a peak after 8 h and then slowly diminished, with a half life of 2.9 days. Of the administered radioactivity, more than 34% was excreted in the urine, mainly as glucosamine, and 1.7% was excreted in the feces. Radioactivity was excreted also as [^{14}C]-CO2 in the expired air. The radioactivity, after i.v. administration, diffused rapidly from blood into the body. Some organs showed an active uptake of radioactivity, e.g. the liver and the kidney. The data also showed that other tissues, such as the articular cartilage, had an active uptake. The authors stated that In most other organs, the radioactivity found could be explained by passive diffusion from plasma. Following oral administration of a single dose of [^{14}C]-glucosamine, the radioactivity was quickly and nearly completely absorbed from the gastrointestinal tract. The pattern of disappearance, metabolic transformation, tissue distribution and excretion of the radioactivity were consistent with those found after i.v. administration [Setnikar, *et al.,* 1986 Vol 12 Tab 233].

The pharmacokinetics of glucosamine sulfate (CAS 29031-19-4) was investigated by the same laboratory in 6 healthy male volunteers (2 per administration route) using ^{14}C uniformly labeled glucosamine sulfate and administering it in single doses by either an intravenous (i.v.), an intramuscular (i.m.) or an oral route. The results showed that after i.v. administration, the radioactivity due to glucosamine could be detected in plasma and was rapidly eliminated, with an initial half life of 0.28 hours. 1-2 hours after administration, the radioactivity due to

glucosamine disappeared almost completely, and was replaced by radioactivity originating from plasma proteins, in which glucosamine or its metabolites were incorporated. This radioactivity reached a peak after 8-10 hours and then declined with a half life of 70 hours. About 28% of the administered radioactivity was recovered in the urine 120 hours following the administration, and less than 1% was recovered in the feces. After i.m. administration similar pharmacokinetic patterns were observed. After oral administration a proportion close to 90% of glucosamine sulfate was absorbed. Free glucosamine was not detectable in plasma. The radioactivity incorporated in the plasma proteins followed pharmacokinetic patterns, which were similar to those after i.v. or i.m. administration, but the concentration in plasma was about 5 times less than after parenteral administration. The counts after oral administration were 26% of that after i.v., or i.m. administration. The authors hypothesized that the lower levels of plasma radioactivity after oral administration were probably due to a first pass effect in the liver, which metabolizes a notable proportion of glucosamine into smaller molecules and ultimately into CO2, water and urea [Setnikar, *et al.,* 1993 Vol 12 Tab 235].

In another study Setnikar *et al.,*1991, found that oral daily doses of glucosamine sulphate of 50-800 mg/kg were effective in assisting with symptoms of arthritis. The authors stated that the potency of glucosamine sulphate in comparison of that of the indomethacin used as a reference substance was found 50-300 times lower. However, since the toxicity of indomethacin in chronic toxicity experiments has been reported to be 1000-4000 times larger, the therapeutic margin with regard to prolonged treatments of inflammatory disorders results in a margin of safety, which is 10-30 times more favorable for glucosamine sulphate than for indomethacin [Setnikar *et al.,*1991 Vol 12 Tab 234].

2. Chitosan Degradation

The initial degradation rates [r] in human serum of three chitosans characterized by varying fractions of N-acetylated units (F_A) were determined by measuring the decrease in viscosity as a function of time. The F_A of the three chitosans was 0.42, 0.5 1, and 0.60 respectively. A strong increase in r with increasing F_A of the chitosans was observed. The degradation rate increased proportionally to $F_A^{4.5}$. With increasing concentrations of lysozyme added to the reaction mixtures of chitosan and serum, the relative increase in degradation rate of chitosans with increasing F_A was almost the same as that without lysozyme added. Addition of the chitinase inhibitor allosamidin

[50 μM] did not inhibit the degradation rate of chitosan [F_A = 0.60] by human serum. The results suggested that chitosans are actually mainly depolymerized by lysozyme in human serum, and not by other enzymes or other depolymerization mechanisms [Varum, *et al.,* 1997 Vol 11 Tab 213].

Chitin was deacetylated to various extents with NaOH to obtain partially and thoroughly deacetylated chitins. The specimens used in this study were deacetylated by 0 [chitin], 68.8, 73.3, 84.0, 90.1 and 100 mol% [chitosan]. Films with a thickness of 150 microns were prepared from these specimens by the solution casting method. The equilibrated water contents of the films were 52.4 [chitin], 73.8 [68.8 mol%], 64.2 [73.3 mol%], 61.8 [84.0 mol%], 57.8 [90.1mol%] and 49.7 wt% [chitosan], while the tensile strengths of the water swollen films were 244 [chitin], 197 [68.8 mol%], 232 [73.3 mol%], 320 [84.0 mol%], 293 [90.1 mol%] and 433 g mm-2 [chitosan]. The maximum water content and the minimum tensile strength observed for a specimen deacetylated between 0 and 68.8 mol% may be ascribed to the lowered crystallinity by deacetylation of chitin, since both chitin and chitosan are crystalline polymers. Unlike their physical properties, *in vitro* and *in vivo* degradations of these films occurred less rapidly without passing a maximum or minimum, as their degree of deacetylation became higher. The *in vitro* degradation was carried out by immersing the films in buffered aqueous solution of pH 7 containing lysozyme at 37º C, while the *in vivo* degradation was studied by subcutaneously implanting the films in the back of rats. It was found that the rate of *in vivo* biodegradation was very high for chitin and 68.8 mol% deacetylated chitin, compared with that for the 73.3 mol% deacetylated chitin. The films, which were more than 73.3 mol% deacetylated, showed slower biodegradation. Interestingly, the tissue reaction towards highly deacetylated derivatives including chitosan was very mild, although they had cationic primary amines in the molecule [Tomihata, *et al.*, 1997 Vol 9 Tab 163].

3. Chitosan Effects Adherence on Dental-Caries-Causing Bacteria

It was previously shown that a low-molecular-weight chitosan and its derivatives N-carboxymethyl chitosan and imidazolyl chitosan inhibit *Streptococcus mutans* adsorption to hydroxyapatite. The ability of the same molecules to interfere with adhesive properties of other oral streptococci [*Streptococcus sanguis, Streptococcus gordonii, Streptococcus constellatus, Streptococcus anginosus, Streptococcus intermedius, Streptococcus oralis, Streptococcus salivarius, Streptococcus vestibularis*] was tested. When saliva coated or

uncoated hydroxyapatite beads were treated with N-carboxymethyl chitosan, a reduction varying from 60% to 98% depending on strains was observed. Low-molecular-weight chitosans and imidazolyl chitosan did not have any effect. Growth in N-carboxymethyl chitosan-supplemented medium [final concentrations ranging from 20 to 500 micrograms/ml-1] caused a dose related reduction in the adsorption of all strains to hydroxyapatite in their affinity towards xylene. No effect was observed with low- molecular weight chitosans and imidazolyl chitosan. In contrast to what was observed with *S. mutans*, the three polysaccharides did not affect detachment from hydroxyapatite beads and adherence to cheek epithelial cells of the other streptococci. These results suggest that low- molecular-weight chitosans and/or imidazolyl chitosan, selectively effect *S. mutans* adsorption to hydroxyapatite, making chitosans very interesting as potential anti-dental caries agents [Tarsi, *et al.,* 1998 Vol 9 Tab 161].

The effects of low molecular weight chitosan [LMCS] on pH responses of human dental plaque following exposure to fermentable carbohydrates were investigated by an ion-sensitive field-effect transistor electrode system. After the plaque pH values were minimized by direct application of 5% glucose solution or consumption of sugared caramel, the subjects started chewing the test gums containing 0 [control], 1 or 3% [w/w] LMCS for three minutes. The pH response was monitored until it recovered to over pH 5.5. In the case of the glucose solution, chewing 3% LMCS gum caused significantly more rapid pH recovery toward the resting level than did the control gum. Initial pH rising rate during gum chewing was faster with either of the two LMCS gums than with the control gum. In the case of caramel, additional effects of LMCS were observed numerically as LMCS content increased. The findings indicated that LMCS had a potential to promote recovery of plaque pH after acidogenic challenge and to maintain the plaque pH around neutrality [Shibasaki, *et al.,* 1994 Vol 8 Tab 148].

4. Antigenotoxic Activity of Chitosan

The antigenotoxic activities of chitin and chitosan were studied using a sister chromatid exchange assay by examining the adsorption of four kinds of mutagens. These two dietary fibers of animal origin showed similar patterns in reducing the genotoxicity of aqueous solutions of the hydrophobic mutagens, 4-nitroquinoline-N-oxide and dinitropyrene, in distilled water. Under similar conditions, the antigenotoxic activities of chitin and chitosan for mitomycin C (MMC) were 87 and 0%, and those for adriamycin (ADM) were 47 and 78%, respectively. In addition, the

antigenotoxic activity of both fibers for MMC was affected by the pH value of the aqueous solution, between 2.5 and 7.2, but that of ADM was not. The results demonstrated that chitin and chitosan may have protective effects against environmental mutagens by adsorbing them in ionic and nonionic solutions [Ohe, 1996 Vol 7 Tab 111].

The effect of dietary chitosan, a fiber of animal origin, on the induction and development of colonic precursor lesions was determined in CF1 mice by Torzsas, *et al.,* 1996. Over a two-week pretreatment period, one group of mice was given azoxymethane [four injections of 5 mg/kg body weight] and the other was treated with saline. Subsequent to carcinogen or saline pretreatment, mice were randomized into one of three dietary groups: [i] control AIN-76 diet; [ii] control AIN-76 diet with 2% low molecular weight chitosan [LMWC]; or [iii] control AIN-76 diet with 2% high molecular weight chitosan [HMWC]. Mice were maintained on this diet for 6 wks. Mice fed diet containing HMWC had significant reductions in the number of aberrant crypt foci, cell proliferation, crypt height and crypt circumference in the colonic epithelium [$P < 0.05$]. In mice given a diet supplemented with LMWC there was a trend towards reduced numbers of aberrant crypts, as well as decreased crypt height and circumference. The results of this study indicated that dietary chitosan reduced the incidence of early preneoplastic markers of colon carcinogenesis, as indicated by aberrant crypts and mitotic figures and altered crypt morphometrics in the murine colon. The authors indicated that molecular range and viscosity of the chitosan might influence the efficacy of this activity [Torzsas. *et al.,* 1996 Vol 9 Tab 164].

H. Other Regulatory Approvals

Chitosan is approved as a food in Japan, and several products ranging from biscuits to fishcakes have been approved by the Japanese Ministry of Agriculture, Fisheries and Foods as "Foods for Specified Health Use" (FOSHU) [FOSHU, 1999 Vol 7 Tab 108]. Foods bearing a FOSHU approval are considered to improve the diet and to help maintain or improve health, when consumed in the normal way. Japan is the world's leader in the manufacture of chitosan, accounting for 90% of the global chitosan market worth approximately 4 trillion yen (120 yen = 1 US dollar) [*Korea Times*, April 7, 1999 Vol 12 Tab 236]. Nearly 50% of the chitosan manufactured in Japan is used in purifying drinking and wastewater streams. The *Korea Times* reports that there are ten firms manufacturing chitosan in Japan, and that 60 package chitosan into various products [*Korea Times*, April 7, 1999 Vol 12 Tab 236].

Chitosan has also been approved for use in Korea, and appears in that country as a food for the improvement of the health of the digestive tract [Korean Society for Chitin and Chitosan, 2000 Vol 6 Tab 80]. Five firms are reported to manufacture chitosan in Korea, and 70 companies make end products using chitosan [*Korea Times*, April 7, 1999 Vol 12 Tab 236].

Recently, the United States Environmental Protection Agency (EPA) has exempted poly-D-glucosamine (chitosan) from the requirement of a tolerance [Federal Register Vol 7 Tab 108]. Under the Federal Food, Drug, and Cosmetic Act, the biochemical growth regulator poly-D-glucosamine (chitosan) is exempt from the requirement of a tolerance for residues when used as a seed treatment in or on rice. The Agency stated that based on the nontoxic nature of the chemical, the EPA was also establishing an exemption from the requirement of a tolerance for residues of poly-D-glucosamine (chitosan) when used as a pesticide in the production of any raw agricultural commodity.

The Australian Complementary Medicines Evaluation Committee considered the use of chitosan under Section 7 of the *Therapeutic Goods Act 1989* in several meetings from June 1998 through September 1998. Under Section 7 the Australian government may declare certain goods to be therapeutic goods when used, advertised, or presented for supply in a particular way. Members of the committee considered data and information, both pro and con, regarding the consumption of chitosan by Australians at doses of 1-2 grams per day. Recommendations to the committee included the following:

1. Chitosan is suitable for use as an active ingredient in Listable products without limits.
2. A warning should be required on the effect of chitosan on the absorption of other drugs (subject to advice from a chemist) and a statement advising that the product of seafood origin.
3. Seek another round of public comment on the proposal that fiber in capsule, tablet or pill form be declared to be therapeutic goods, under section 7 of the Act.

The reasons for these recommendations were as follows:

a) Chitosan does not appear to present a significant risk to consumers in the manner and dosage likely to be consumed.
b) Chitosan can already be consumed in foods and no evidence is available to date to suggest this poses any safety risk.
c) Sufferers of seafood allergy would be alerted to any dangers if they were aware that chitosan is derived from seafood. A warning statement is not necessary, provided that the product contains a statement "is derived from seafood."

At their final meeting on the topic, members concluded that therapeutic goods containing chitosan should contain dosage directions on the label so that consumers could avoid any interaction between chitosan and prescription drugs.

I. Summary

Chitosan is a natural material that has been consumed by man for thousands of years. The human body treats chitosan as a dietary fiber, utilizing existing enzyme systems for its digestion, and fermenting the remainder by means of indigenous bacteria in the colon. Breakdown products from chitosan include chitooligosaccharides and ultimately glucosamine, which is a normal constituent of the human body. Multiple animal and human studies conducted over a period of time by scientists in various parts of the world have confirmed that chitosan is safe for consumption, and elicits no toxicological effects. Previous concerns about the safety of chitosan consumption by humans have been expressed by Koide, 1998 [Koide, 1998 Vol 6 Tab 78]. Those concerns are shown below [Koide, 1998 Vol 6 Tab 78]. Primex Ingredients ASA has researched these concerns and has provided information in *italics* to support their conclusion that Primex chitosan can be considered generally recognized as safe, when manufactured and used in accordance with current Good Manufacturing Practices.

J. General

"A comprehensive study of the metabolic effects of long-term dietary supplement use of chitosan in human subjects needs to be preformed."

The author may have been unaware of the several human studies listed above in Table 6, in which chitosan was given to a variety of human subjects, both healthy and with renal failure for periods up to 12 weeks. No statistically adverse toxicological events were observed, at the doses given. Doses ranged from 1.0 gram per day to 3-6 grams per day [Jing et al., *1997 Vol 5 Tab 67, Pittler,* et al., *1999 Vol 7 Tab 117, and Maezaki,* et al., *1993, Vol 6 Tab 98].*

K. Bones and Minerals:

"The marked impediment in the absorption of minerals and the reduction in mineral content of bones during the short duration (2 weeks) of chitosan administration suggest a potentially hazardous outcome following prolonged ingestion of the food supplement. It may precipitate an early onset of osteoporosi and/or may aggravate an

existing condition. Calcium supplement should be mandatory in post-menopausal women and elder males. The reduced absorption of the fat-soluble vitamins notably vitamin D will contribute to the calcium deficiency. The absorption of selenium and magnesium may be reduced after prolonged ingestion of the food supplements. These materials are essential elements for the proper metabolism of cells. Although after short-term ingestion of chitin-chitosan, zinc absorption, and bone content remain unchanged, the effect of prolonged consumption on zinc metabolism should be studied."

An in vivo study conducted by Colombo, et al., 1996 [Colombo, et al., 1996 Vol 3 Tab 24 and Table 6 above] demonstrated that humans not only tolerated chitosan administration, but maintained normal blood mineral levels as well. Several experts have pointed out that chitosan does not bind Ca^{++}. They do not agree with the statement regarding mandatory calcium supplementation [Drs. Olav Smidsød and P.A. Sandford, June, 2000, personal communication].

L. Vitamin Deficiency

"Reduced vitamin E intake may have adverse metabolic consequences. Vitamin D and E should be taken with this food supplement. Subjects should be evaluated periodically for signs and symptoms of fat-soluble vitamin deficiency."

Both Colombo, et al., 1995, and Pittler et al., 1999 demonstrated that humans consuming either 1,600 mg or 1.0 gram of chitosan for 4 weeks showed no significant differences in the fat soluble vitamins A, D, or E [Colombo, et al., 1996 Vol 3 Tab 24 and Pittler et al., 1999 Vol 7 Tab 117].

M. Growth Retardation

"Growth of animals is retarded when fed high doses of chitin-chitosan. Ingestion of "fine particles" grade of chitosan is potent at a low dose and its effect occurs within a short period. The effects of these food supplements on the growth of children and functions of aged subjects should be carefully monitored."

The author is referring to studies by Tanaka, et al., *1997 and Razdan,* et al., *1997, in which mice and chickens were fed either 5.0% of their diet, or 30 g/kg chitosan [Tanaka,* et al., *1997 Vol 9 Tab 160 and*

Razdan, et al., *1997 Vol 8 Tab 133]. These results are not surprising, given that 5.0% of a human diet, would be the equivalent of approximately 75 grams per day, and 30 g/kg would equal approximately 1,500 grams per day. Under both circumstances, the caloric content of the diet would be reduced, as it would with any other dietary fiber [Enns,* et al., *1997 Vol 4 Tab 36].*

N. Pregnancy

> "Because chitin-chitosan effects bone metabolism by reducing calcium and possibly vitamin D absorption, partaking of this food supplement by pregnant women may be a potential risk."

As mentioned above, actual studies conducted by several investigators with human volunteers have not demonstrated abnormal mineral, vitamin, or blood chemistry [Colombo, et al., 1996 Vol 3 Tab 24 and Pittler et al., 1999 Vol 7 Tab 117].

O. Malabsorption Syndrome:

> "The ingestion of chitosan at a dose of 3-6 g/day in the form of biscuits by healthy males for 2 weeks was well tolerated, without the development of diarrhea or constipation (6). However chitin-chitosan forms gels in the intestinal tract that entrap lipids, stimulating a mild malabsorption syndrome or sprue-like condition. Although bouts of diarrhea or constipation have not been observed in experimental animals, they may occur in human subjects (2). Subjects with underlying or latent conditions of the malabsorption syndrome or steatorrhea should avoid this food supplement since it may aggravate their symptoms."

Table 6 above summarizes results from a number of human studies, in which stress conditions were induced, due to caloric restrictions. While certain gastrointestinal symptoms, including constipation and nausea were reported in a small percentage of subjects, placebo controlled double blind studies demonstrated that the occurrence of those symptoms were not significantly different from the controls [Veneroni, 1996, Vol 11 Tab 214, and Guistina et al., 1995, Vol 4 Tab 43].

Primex Ingredients ASA suggests that concerns that chitosan may reduce vitamin and mineral absorption have now been addressed by

the publication of several placebo controlled double blind human studies, which have demonstrated that the use of chitosan as a supplement to the diet caused no harm and did not effect vitamin or mineral metabolism *in vivo.*

Many other publications suggesting the safe use of chitosan for various functions internal to the human body, demonstrate that chitosan enjoys a general recognition of its safety by scientists and regulatory officials worldwide. Therefore Primex Ingredients ASA submits that its chitosan ingredient can be considered Generally Recognized as Safe, when manufactured and used according to current Good Manufacturing Practices.

Primex Chitosan Expert Panel Opinion

Introduction

An expert panel qualified by scientific training and experience to evaluate the safety of food and food ingredients was assembled to conduct an independent critical evaluation of the safety of Primex chitosan as a food ingredient when produced and used in accordance with current Good Manufacturing Practices and meeting the specifications described herein. The panel members included:

Dr. Joseph F. Borzelleca	Pharmacologist and Toxicologist, Chairman
Dr. Bert N. LaDu, Jr.	Pharmacologist
Mr. Cleve Denny	Microbiologist
Dr. Paul A. Sandford	Chemist, Applications Consultant
Dr. Olav Smidsrød	Chitosan and Polysaccharide Chemist

The qualifications of the members of the Scientific Expert Review Panel (the Panel) are provided in their curricula vitae, which are on file at the offices of the Norwegian Trade Council, 800 Third Avenue, 23rd Floor, New York, New York, 10022 USA. The Panel's qualifications in their respective scientific fields meet the requirements set forth in the Federal Food, Drug, and Cosmetic Act's definition of generally recognized as safe (GRAS) substances (§ 201(s)) and 21 CFR 170.30(a) "Eligibility for classification as generally recognized as safe (GRAS)".

The Panel was charged by Primex Ingredients ASA to critically evaluate the information and data regarding chitosan in general and Primex chitosan specifically. The Panel would then render an opinion, based on scientific principles, on the generally recognized as safe (GRAS) status of Primex chitosan. The opinion of the Panel is based on the general use of Primex chitosan in food when manufactured in accordance with U.S. current Good Manufacturing Practices (21 CFR Part 110) and meeting the specifications provided in the report.

Independently, members of the Panel critically evaluated the results of a literature review on chitosan, information provided by Primex Ingredients ASA, and other pertinent materials. The Panel conferred by telephone and then met in New York City on June 8, 2000 with representatives of Primex Ingredients ASA, who reviewed the pertinent safety and functionality information associated with Primex chitosan. This information was compiled into a twelve-volume report, which supported the eligibility of Primex chitosan as a GRAS ingredient in accordance with 21 CFR 170.30. The information provided for the Panel's review was presented in the form stipulated in 21 CFR 170.35.

Conclusion

Based on the Panel's independent and collective critical evaluation of all the information summarized above, it was determined that Primex chitosan can be considered generally recognized as safe (GRAS), when produced and used in accordance with cGMP as a food in the various major food categories as listed by the United States Department of Agriculture in the *Continuing Survey of Food Intake by Individuals* (CSFII), and when meeting the specifications described herein.

Signatures

Dr. Joseph F. Borzelleca, Chairman — Date: 16 November 2000

Cleve Denny — Date: 16 November 2000

Dr. Bert N. La Du, Jr. — Date: Nov. 21, 2000

Dr. Paul A. Sandford — Date: 11-15-00

Dr. Olav Smidsrød — Date: 14/11-00

In reaching this conclusion, the Panel relied on the following information: (References refer to those cited in the 12-volume GRAS Report)

Chemical Identity and Manufacturing

- Chitosan is a polysaccharide, composed mainly of glucosamine, 2-amino-2-deoxy-β-D-glucose [Shahidi, *et al.,* 1999 Vol 8 Tab 145].

- Chitosan occurs widely in nature in the cell walls of fungi, and as a breakdown product of chitin [Shahidi, *et al.,* 1999 Vol 8 Tab 145].

- Commercially, chitosan is prepared by deacetylating chitin, which is the second most abundant natural polymer on earth [Shahidi, *et al.,* 1999 Vol 8 Tab 145].

- Chitosan has three functional groups, an amino group, as well as both primary and secondary hydroxyl groups, which are the basis for its functionality in foods [Shahidi, *et al.,* 1999 Vol 8 Tab 145].

- Primex Ingredients ASA (Primex) of Avaldsnes, Norway has developed a unique process to manufacture chitosan from the arctic shrimp, *Pandalus borealis.*

- The Primex Ingredients ASA chitosan plant has a certified Hazards Analysis Critical Control Point Program (HACCP) in place to assure product quality. [Primex Ingredients Analytical Methods Vol 7 Tab 118].

- Final product specifications for the various Primex chitosan products have been provided.

- An analysis of five lots of Primex chitosan has demonstrated that the products can consistently meet their published specifications.

- Chemical and heavy metals analyses of Primex chitosan products have shown that the products contain no toxicants of concern. Primex chitosan contains less chemical and microbiological contaminants than many common food sources.

Use and Functionality

- Because of the high cost of producing chitosan, it has not been historically used as a food or food ingredient [Shahidi, *et al.,* 1999 Vol 8 Tab 145].

- Modern humans continue to consume small quantities of chitosan as part of a normal diet. Natural sources of chitosan in the diet include various food fungi, e.g. *Pleurotus ostreatus*, and certain invertebrate animals [Illum, 1998 Vol 5 Tab 61, Chobot, *et al.,* 1995 Vol 3 Tab 22, and Muzzarelli, 1997, Vol 7 Tab 104].

- Applications for chitosan include general use in foods as a multiple-use direct additive.

- Chitosans have been found to have many beneficial properties of use to the food industry, including fat absorption, protein aggregation, emulsification capability, film-forming ability, clarifying ability and antimicrobial activity [Knorr, 1991 Vol 6 Tab 76 and Shahidi *et al.,* 1999 Vol 8 Tab 145].

- Many food scientists have considered chitosan a source of dietary fiber [Furda, 2000, Vol 4 Tab 40, and Dreher, 1987 Vol 4 Tab 27].

Self-Limiting Use in Foods

- Studies by food scientists have shown that chitosan has been an effective emulsifier at percentages of use of less than 1.0% [Poole, 1988 Vol 11 Tab 225 and Rodriguez, *et al.,* 2000 Vol 12 Tab 237].

- Use of chitosan is expected to be limited by its physicochemical properties.

- Excessive chitosan usage may produce aggregation of ingredients, depending on the synergies involved and the pH of the formulation [Poole, 1988 Vol 11 Tab 225]. Poole found that use of greater than 0.8% chitosan yielded a dense aggregate in a whey protein isolate-corn oil emulsion [Poole, 1988 Vol 11 Tab 225].

Exposure

- Primex Ingredients ASA has provided an estimate of exposure to its ChitoClear™ chitosan products. Additional exposure to chitosan may result from its use as a source of dietary fiber in dietary supplement form [Enns, *et al.,* 1997 and Langhelle, 1999 Vol 8 Tab 126].

- The mean and the 90th percentile of estimated exposure to Primex ChitoClear™ chitosan assume that 100% of consumers are "eaters" of chitosan-containing products within a given food category, and that all foods within a given category contain chitosan.

- The mean and the 90th percentile of estimated exposure to Primex ChitoClear™ chitosan (7.97 and 15.94 grams respectively) remains lower than the recommended levels of intake for dietary fiber (25-30 grams per day) in the United States [Enns, *et al.,* 1997 Vol 4 Tab 36].

- The recommended dose of chitosan as a dietary supplement is 1.5 to 3.0 grams per day. This dosage has been recommended based on the results of clinical trials with human volunteers [Colombo, *et al.*, 1996, Vol 3 Tab 24].

Current Usage

- One company, Vanson Incorporated, Redmond, WA, manufactures chitosan in the United States.

- Chitosan is approved as a food in Japan, and several products ranging from biscuits to fishcakes have been approved by the Japanese Ministry of Agriculture, Fisheries and Foods as "Foods for Specified Health Use" (FOSHU) [FOSHU, 1999 Vol 7 Tab 108].

- Japan is the world's leader in the manufacture of chitosan, accounting for 90% of the global chitosan market, worth approximately 4 trillion yen (US$ 33.3 billion, where 120 yen = 1 US dollar) [*Korea Times*, April 7, 1999 Vol 12 Tab 236].

- The *Japan Times* of April 11, 2000 reported that sales of dietary chitosan in Japan totaled nearly US$1 billion per year [*Japan Times,* April 11, 2000 Vol 7 Tab 108].

- A 1999 US State Department Document entitled "Japan – Chitin and Chitosan Market – IMI990408" indicated that the current health food market for chitosan in Japan is slightly more than 200 metric tons per year.

- Ten firms are reported to manufacture chitosan in Japan, and 60 are reported to package chitosan into various products [*Korea Times*, April 7, 1999 Vol 12 Tab 236].

- Chitosan has also been approved for use in Korea, and appears in that country as a food for the improvement of the health of the digestive tract [Korean Society for Chitin and Chitosan, 2000 Vol 6 Tab 80].

- Five firms are reported to manufacture chitosan in Korea, and 70 companies make end products using chitosan [*Korea Times*, April 7, 1999 Vol 12 Tab 236].

- The Australian Complementary Medicines Evaluation Committee considered the use of chitosan under Section 7 of the *Therapeutic Goods Act 1989* from June 1998 through September 1998. Members of the committee considered data and information, both pro and con, regarding the consumption of chitosan by Australians at doses of 1-2 grams per day, and concluded that Chitosan does not appear to present a significant risk to consumers in the manner and dosage likely to be consumed.

- Use of chitosan as a dietary supplement in the U.S. has been growing rapidly. According to Information Resources, Inc., approximately US $6 million has been sold by the three leading brands since February, 1999. This translates to approximately 240,000 kilograms of consumption, at an average wholesale price of U.S. $25 per kilogram. Actual usage is now estimated to be 500,000 kilograms

per year, since the *Natural Products Insider, 1999 Buyer's Guide* listed 72 suppliers/distributors of chitosan in the US.

- Recently, the United States Environmental Protection Agency (EPA) has exempted poly-D-glucosamine (chitosan) from the requirement of a tolerance [Federal Register Vol 7 Tab 108]. Chitosan is suitable for use as an active ingredient in listable products without limits.

Animal Studies

- Thirty-three animal safety and functionality studies in five species of animals were considered by the Panel.

- Both human and animal studies have shown that chitosan is well tolerated, and that its metabolism produces substances that are found in the human body and other dietary substances [Kelly, 1998 Vol 12 Tab 226].

- A contact hypersensitivity test with albino guinea pigs showed that none of the control and test animals were observed to have skin reactions after the challenge treatment with Primex chitosan at a concentration of 1.875 %.

Human Safety

- Ten published human studies conducted in various countries with volunteers consuming 1.0 – 6.0 grams of chitosan per day demonstrated that vitamin, mineral, and hemoglobin balances remained within normal limits.

- Adverse events accompanying the use of chitosan and a calorie-restricted diet included mild and transitory nausea, flatulence, or constipation in < 5% of the subjects. However, there was no statistically significant difference in the number or severity of adverse events between the treatment and control groups [Veneroni, *et al.,* 1996 Vol 11 Tab 214, and Sciutto, *et al.,* 1995 Vol 8 Tab 141].

Fate of Chitosan in the Gastrointestinal Tract

- Hirano indicated in 1989 that approximately 60 tons of chitosan per year went into the production of D-glucosamine [Hirano, 1990 Vol 5 Tab 54].

- The metabolic fate of chitosan in the digestive tract of man has been tracked through radiolabelled preparations [Macleod, *et al.,* 1999 Vol 6 Tab 97]. Studies demonstrated that chitosan is not degraded until it reaches the colon [Macleod, *et al.,* 1999 Vol 6 Tab 97].

- The hydrolysis of chitosan has been studied, and the degradation of natural polymers, such as chitosan and chitin proceeds via enzymatic degradation [Domard, *et al.*, 1989 Vol 12 Tab 227 and Lee *et al.*, 1995 Vol 6 Tab 91].

- Both chitin and chitosan have been known to be digestible by chitinase and lysosyme in accordance with the quantity of N-acetyl groups and their distribution within the backbone of the molecule [Lee, *et al.*, 1995 Vol 6 Tab 91, Aiba, 1993 Vol 12 Tab 228, Aiba, 1992 Vol 12 Tab 229, and Sashiwa, *et al.*, 1990 Vol 12 Tab 230].

- Muzzarelli, 1997, has provided evidence for the degradation of chitosan in humans via non-specific chitinase and lysosymal enzyme systems [Muzzarelli, 1997 Vol 7 Tab 104].

- Chitosan is degraded into chitooligosaccharides, and then finally into glucosamine [Domard, 1989, Vol 12 Tab 227, Aiba, 1993 Vol 12 Tab 228, and Aiba, 1992 Vol 12 Tab 229].

- Glucosamine, which is the final break-down product of chitosan is also formed in the human body as glucosamine 6-phosphate (G6-P) [Kelly, 1998 Vol 11 Tab 226].

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End Submission

AM

LEE B. DEXTER & ASSOC.
TECHNOLOGY CONSULTANTS

15704 WEBBERVILLE ROAD
AUSTIN, TEXAS 78724 USA

TELEPHONE (512) 276-7408
FAX (512) 276-7489

March 15, 2001

Dr. Linda Kahl
Office of Premarket Approval
Center for Food, Safety and Nutrition HFS 200
Food and Drug Administration
200 C St. SW
Washington, DC 20204
Via Fax: (202) 418-3131

Confirmation via US Mail

Re: Clarification of the Availability of Data and Information For the Primex Chitosan GRAS Notification

Dear Dr. Kahl:

Thank you for your prompt attention to the GRAS Notification submitted by Primex Ingredients ASA (Primex) of Avaldsnes, Norway in support of shrimp-derived chitosan. The purpose of this letter is to provide a clarification regarding the availability of data and information for the Notification. The data and information will be available at the address of the notifier (listed below), and the notifier will promptly supply any information requested by the Office of Premarket Approval. Primex would be happy to supply any information based on requests by telephone or in writing. If requests are made by telephone, a follow-up written copy of the request would be appreciated.

Primex Ingredients ASA
% Norwegian Trade Council
800 Third Avenue, 23rd Floor
New York, New York, USA 10022
Telephone: (212) 421-9210
Fax: (212) 838-0374

Again, thank you very much for your attention to this matter. If you have any questions regarding the content of the Notification, you may reach me at the number listed above.

Sincerely,

Lee B. Dexter
Technical Consultant

CC:
Mr. Steven Dillingham, Norwegian Trade Council
Mr. Bjarte Langhelle, Primex
Mr. Thore Ivar Thorsen, Primex
Mr. Sigurd Mølstre, Primex

AM

LEE B. DEXTER & ASSOC.
TECHNOLOGY CONSULTANTS

15704 WEBBERVILLE ROAD
AUSTIN, TEXAS 78724 USA

TELEPHONE (512) 276-7408
FAX (512) 276-7489

MEMORANDUM

TO: DR. KATHLEEN MCAVENEY JONES, CFSAN

FROM: LEE B. DEXTER PAGES: 3

DATE: MAY 22, 2001

RE: REPLACEMENT STRUCTURE FOR CHITOSAN IN GRN 73

LEE B. DEXTER & ASSOC.
TECHNOLOGY CONSULTANTS

15704 WEBBERVILLE ROAD
AUSTIN, TEXAS 78724 USA

TELEPHONE (512) 276-7408
FAX (512) 276-7489

May 18, 2001

Dr. Kathleen McAveney Jones
Consumer Safety Officer
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Premarket Approval
Division of Product Policy, HFS-206
200 C Street, S.W.
Washington, DC 20204
Fax: (202) 418-3131

Dear Dr. McAveney Jones

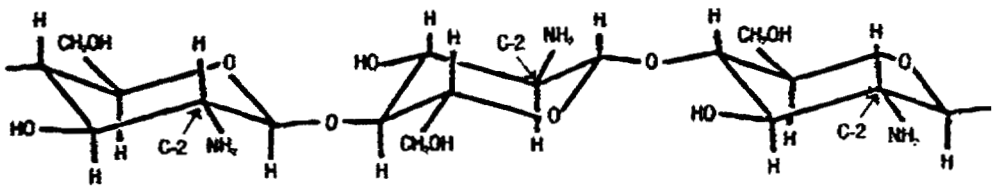
Re: Replacement Structure for Chitosan in GRN: 73

Thank you for your telephone call regarding the need to replace the schematic for the structure of chitosan in GRAS Notice number 73. Please find a correct structure attached. The reference for this structure is: Sandford, P.A., 1989, Chitosan: Commercial Uses and Potential Applications in Chitin and Chitosan, Skjåk-Bræk, G., Anthonsen, A., and Sandford, P., Eds. Elsevier Applied Science, London and New York. This structure will replace the one found on page 7, no. 2 in GRAS Notice 73.

Again, we apologize for the confusion. Please don't hesitate to call if this schematic does not meet the needs of the chemistry reviewers.

Sincerely,

Lee B. Dexter
Technical Consultant



(b) CHITOSAN

000254

AM

LEE B. DEXTER & ASSOC.
TECHNOLOGY CONSULTANTS

15704 WEBBERVILLE ROAD
AUSTIN, TEXAS 78724 USA

TELEPHONE (512) 276-7408
FAX (512) 276-7489

MEMORANDUM

TO: DR. KATHLEEN MCAVENEY JONES
VIA FAX: (202) 418-3131

FROM: LEE B. DEXTER

DATE: OCTOBER 29, 2001

RE: SUPPLEMENT FOR CHITOSAN

Dear Dr. McAveney Jones:

Thank you for the very helpful meeting last week on the isoamylase submission. Please find attached below, an airbill for FedEx, for the chitosan supplement. You should receive it tomorrow, October 30th. I thought it might be helpful, if you had the tracking information ahead of time. Thank you for all your assistance with this supplement. It has been most appreciated.

000259

FedEx Express USA Airbill Tracking Number 828498220079

1 From Please print and press hard
Date 10/29/01 Sender's FedEx Account Number
Sender's Name Lee B. Dexter Phone (512) 276-7408
Company Dexter & Assoc.
Address 15704 Webberville Rd. Dept./Floor/Suite/Room
City Austin State TX ZIP 78724

2 Your Internal Billing Reference
First 24 characters will appear on invoice.

3 To
Recipient's Name Dr. McAveney Jones Phone (202) 418-3097
Company OFFICE of FOOD ADDITIVE SAFETY
Address DIV of BIOTECH + GRAS NOTICE REVIEW, HFS, 206, 200 C ST. SW
To "HOLD" at FedEx location, print FedEx address. We cannot deliver to P.O. boxes or P.O. ZIP codes. Dept./Floor/Suite/Room
City WASHINGTON State DC ZIP 20204

Questions? Visit our Web site at fedex.com or call 1-800-Go-FedEx® (800)463-3339.

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Sender's Copy

4a Express Package Service — Packages up to 150 lbs.
Delivery commitment may be later in some areas.
- [ ] FedEx Priority Overnight Next business morning
- [X] FedEx Standard Overnight Next business afternoon
- [ ] FedEx First Overnight Earliest next business morning delivery to select locations
- [ ] FedEx 2Day Second business day
- [ ] FedEx Express Saver Third business day
- [ ] NEW FedEx Extra Hours Later drop-off with next business afternoon delivery to select locations

FedEx Envelope rate not available. Minimum charge. One-pound rate

4b Express Freight Service — Packages over 150 lbs.
Delivery commitment may be later in some areas.
- [ ] FedEx 1Day Freight* Next business day
- [ ] FedEx 2Day Freight Second business day
- [ ] FedEx 3Day Freight Third business day

* Call for Confirmation:

5 Packaging — * Declared value limit $500
- [X] FedEx Envelope*
- [ ] FedEx Pak* Includes FedEx Small Pak, FedEx Large Pak, and FedEx Sturdy Pak
- [ ] Other Pkg. Includes FedEx Box, FedEx Tube, and customer pkg.

6 Special Handling — Include FedEx address in Section 3.
- [ ] SATURDAY Delivery RESTRICTIONS Available only for FedEx Priority Overnight and FedEx 2Day to select ZIP codes
- [ ] SUNDAY Delivery RESTRICTIONS Available only for FedEx Priority Overnight to select ZIP codes
- [ ] HOLD Weekday at FedEx Location RESTRICTIONS Not available with FedEx First Overnight
- [ ] HOLD Saturday at FedEx Location RESTRICTIONS Available only for FedEx Priority Overnight and FedEx 2Day to select locations

Does this shipment contain dangerous goods?
- [X] No
- [ ] Yes As per attached Shipper's Declaration
- [ ] Dry Ice Dry Ice, 9, UN 1845 ____ x ____ kg
- [ ] Cargo Aircraft Only

Dangerous Goods cont. Dry Ice cannot be shipped in FedEx packaging or via FedEx Extra Hours service.

7 Payment Bill to:
- [ ] Sender Acct. No. in Section 1 will be billed
- [ ] Recipient
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- [ ] Credit Card
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FedEx Acct. No. Credit Card No. Exp. Date

Total Packages	Total Weight	Total Declared Value†
		$ .00

†Our liability is limited to $100 unless you declare a higher value. See back for details. FedEx Use Only

8 Release
By signing you authorize us to deliver this shipment without obtaining a signature and agree to indemnify and hold us harmless from any resulting claims.

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AM

LEE B. DEXTER & ASSOC.
TECHNOLOGY CONSULTANTS

15704 WEBBERVILLE ROAD
AUSTIN, TEXAS 78724 USA

TELEPHONE (512) 276-7408
FAX (512) 276-7489

MEMORANDUM

TO: DR. KATHLEEN MCAVENEY JONES
VIA FAX: (202) 418-3131

FROM: LEE B. DEXTER **PAGES:** 16

DATE: NOVEMBER 2, 2001

RE: GRAS NOTICE 74

Dear Dr. McAveney Jones:

Dr. Kahl has called this afternoon, to let us know that the FedEx containing the amendment for GRAS Notice 74 has not been received, and she also let me know that I did not address it correctly. I apologize for that error. Upon learning that the document was short enough to be faxed, Dr. Kahl suggested that I fax it.

Please find attached:

A letter of Amendment
An Amended GRAS Claim
An Amended Section III (Probable Consumption)
A List of recently identified references

Thank you again, for all your time and attention to this submission. We will follow this fax with either the same package, or a new one addressed correctly, and containing 3 hard copies.

LEE B. DEXTER & ASSOC.
TECHNOLOGY CONSULTANTS

15704 WEBBERVILLE ROAD
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TELEPHONE (512) 276-7408
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October 26, 2001

Dr. Kathleen McAveney Jones
U.S. Food and Drug Administration
Center for Food, Safety and Nutrition
Office of Food Additive Safety
Division of Biotech and GRAS Notice Review, HFS 206
200 C St. SW
Washington, DC 20204

Re: GRAS Notice 74: Shrimp-Derived Chitosan

Dear Dr. McAveney Jones:

In accordance with the proposed rule for Substances Generally Recognized as Safe, which was published in the *Federal Register* at Vol. 62, No. 74 on April 17, 1997, and a teleconference with personnel in the Division of Biotech and GRAS Notice Review, Primex Ingredients (Primex) of Avaldsnes, Norway would like to amend its notice of a claim that the use of shrimp-derived chitosan as a food ingredient is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act.

Specifically, Primex would like to amend the section of its notification covering product exposure. At the suggestion of the agency, Primex has reviewed its original estimate of the use levels of chitosan in food, and has found that the use levels originally put forth were overestimated. This conclusion was based on a survey of the chitosan industry, including chitosan suppliers and users in Asia and Europe, as well as studies on the uses of chitosan films published recently.

Taken together, these data have shown that the majority of chitosan uses in foods involve dilute solutions, or films formed from dilute solutions. Therefore, the actual chitosan solids usage is less than originally estimated. The original estimation of the mean intake was 7.97 grams per day. That value has now been reduced to 1.49 grams per day.

In order to properly amend its submission, Primex is submitting an amended GRAS Claim, which adds a technical effect as an antimicrobial. This is warranted, because many of the uses of chitosan films involve food protection through its antioxidant, and antimicrobial properties. In addition, Primex is amending Section

III of its Notification, which covers probable consumption, and is adding a reference list of recent publications, which support the revised intake estimate.

Also, in order to clarify the source of the chitosan, which is the subject of this Notification, Primex is amending the common or usual name of its chitosan in the amended GRAS Claim. The common or usual name will be Shrimp-Derived Chitosan.

Primex would like to thank the agency for the opportunity to amend its submission, and the company appreciates the agency's patience while this amendment was being compiled. A number of translations were required to develop a more accurate estimate of the quantity of chitosan solids actually being used in food formulations.

In the future Primex will also submit a notice to the agency, regarding a potential name change. Primex has recently merged with an Icelandic company, and the details of a new business organization are still being developed. The U.S. office for Primex will remain at the Norwegian Trade Council, whose address appears below.

Norwegian Trade Council
800 Third Avenue, 23rd Floor
New York, New York, USA 10022
Telephone: (212) 421-9210
Fax: (212) 838-0374

I will be happy to respond to any technical questions regarding this submission, and my contact information is shown on the letterhead.

Again, thank you for the opportunity to amend GRAS Notice 74. Attached to this letter are a revised GRAS Claim, a revised Section III, and a supplemental reference list.

Sincerely,

Lee B. Dexter
Technical Consultant

CC: Dr. Linda Kahl, CFSAN
Mr. Steve Dillingham, Norwegian Trade Council
Mr. Bjarte Langhelle, Primex
Mr. Thore Ivar Thorsen, Primex

Shrimp-Derived Chitosan: GRAS Claim

Introduction

Chitosan is a polysaccharide, composed mainly of glucosamine, 2-amino-2-deoxy-β-D-glucose. Chitosan occurs widely in nature in the cell walls of fungi, and as a breakdown product of chitin. Commercially, chitosan is prepared by deacetylating chitin, which is the second most abundant natural polymer on earth [Shahidi, *et al.*, 1999 Vol 8 Tab 145]. The fact that chitosan is a natural substance, and is derived from a natural substance, allows it to be a candidate for GRAS status as a food ingredient. The structure of chitin is similar to that of cellulose, and chitosan has long been considered a source of dietary fiber, as it cannot be completely broken down in the human digestive tract [Omrod, 1998 Vol 7 Tab 112]. Chitosan has three functional groups, an amino group, as well as both primary and secondary hydroxyl groups, which are the basis for its functionality in foods [Shahidi, *et al.*, 1999 Vol 8 Tab 145].

Primex Ingredients (Primex) of Avaldsnes, Norway has developed a unique process to manufacture chitosan from the arctic shrimp, *Pandalus borealis*. A consistent source of raw material and well designed manufacturing practices have made it possible to use chitosan more widely as a food ingredient. This Notification document contains the information required in proposed 170.36 to allow the FDA to evaluate whether the submitted notice provides a sufficient basis for a generally recognized as safe (GRAS) determination. This document is being submitted by Primex Ingredients of Avaldsnes, Norway.

In compliance with 21 CFR § 170.30, Primex determined that Shrimp-Derived Chitosan can be considered GRAS when used in accordance with current Good Manufacturing Practices. Primex wishes to voluntarily notify the Center for Food Safety and Applied Nutrition (CFSAN) of that determination, and according to proposed § 170.36, the company is submitting the following GRAS exemption claim.

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Primex Ingredients has prepared an eleven-volume GRAS Report, which forms the basis for the information found in this notification. The company also commissioned a panel of experts (Expert Panel), qualified by scientific training and experience to assess the safety of food ingredients, who critically evaluated the chitosan GRAS Report as well as other data and information relevant to the use and safety of this ingredient. In a meeting held on June 8, 2000, the panel concurred with the company's determination that Shrimp-Derived Chitosan can be considered generally

recognized as safe for general use in food. Based on the data and information contained in the Report and the opinion of the expert panel (which is attached to this notification), Primex explicitly accepts responsibility for the GRAS determination of Shrimp-Derived Chitosan, known commercially as ChitoClear™.

Section I. GRAS Exemption Claim

Primex Ingredients hereby notifies the U.S. Food and Drug Administration that the use of Shrimp-Derived Chitosan (as a food ingredient) is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act, because Primex has determined that such use is GRAS.

4. Notifier:

Primex Ingredients
% Norwegian Trade Council
800 Third Avenue, 23rd Floor
New York, New York, USA 10022
Telephone: (212) 421-9210
Fax: (212) 838-0374

5. Common or Usual Name:

Shrimp-Derived Chitosan

3. Applicable Conditions of Use:

Applications for chitosan include general use in foods as a multiple-use direct additive. The ingredient should be used under the conditions of current Good Manufacturing Practice.
In order to classify the various effects ingredients may have in food, FDA has published a list of 32 physical or technical functional effects for which direct food ingredients may be added to food. These are codified at 21 CFR §170.3 (o) (1-32). Applications for chitosan are covered under several of the following terms as listed under 21 CFR §170.3 (o).

(2)"Antimicrobial agents": Substances used to preserve food by preventing growth of microorganisms and subsequent spoilage, including fungistats, mold and rope inhibitors, and the effects listed by the National Academy of Sciences/National Research Council under "preservatives."

Section I Notification Claim (Continued)

(3) "Antioxidants": Substances used to preserve food by retarding deterioration, rancidity, or discoloration due to oxidation.
(5) "Curing and pickling agents": Substances imparting a unique flavor and/or color to a food, usually producing an increase in shelf life stability.
(6) "Dough strengtheners": Substances used to modify starch and gluten, thereby producing a more stable dough, including the applicable effects listed by the National Academy of Sciences/National Research Council under "dough conditioner."
(8) "Emulsifiers and emulsifier salts": Substances which modify surface tension in the component phase of an emulsion to establish a uniform dispersion or emulsion.
(14) "Formulation aides": Substances used to promote or produce a desired physical state or texture in food, including carriers, binders, fillers, plasticizers, film-formers, and tableting aids, etc.
(16) "Humectants": Hygroscopic substances included in food to promote retention of moisture, including moisture-retention agents and antidusting agents.
(20) "Nutrient supplements": Substances which are necessary for the body's nutritional and metabolic processes.
(24) "Processing aids": Substances used as manufacturing aids to enhance the appeal or utility of a food or food component, including clarifying agents, clouding agents, catalysts, flocculants, filter aids, and crystallization inhibitors, etc.
(28) "Stabilizers and thickeners": Substances used to produce viscous solutions or dispersions, to impart body, improve consistency, or stabilize emulsions, including suspending and bodying agents, setting agents, jellying agents, and bulking agents, etc.
(29) "Surface-active agents": substances used to modify surface properties of liquid food components for a variety of effects, other than emulsifiers, but including solubilizing agents, dispersants, detergents, wetting agents, rehydration enhancers, whipping agents, foaming agents, and defoaming agents, etc.
(31) "Synergists": Substances used to act or react with another food ingredient to produce a total effect different or greater than the sum of the effects produced by the individual ingredients.
(32)"Texturizers": Substances which affect the appearance or feel of the food.

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Section I Notification Claim (Continued)

6. Basis of the GRAS Determination

The basis of the GRAS determination for Shrimp-Derived Chitosan was the use of scientific procedures.

7. Availability of Data and Information and Key to References

The data and information that are the basis of the GRAS determination for Shrimp-Derived Chitosan will be available at the address of the notifier listed above. However, throughout this Notification, citations to the published literature, which were included in the 11-volume GRAS Report are denoted as follows: [Author (*et al*), Year, Tab (number) Volume (number)]. In order to facilitate review of this document a complete list of references from the chitosan GRAS Report is included in Appendix 2 as a key. Recently identified references, which were not included in the GRAS Report are shown between parentheses ( ) within the text of this document and are given in a standard bibliographic form.

6. Signature of an official for Primex Ingredients

Official for Primex Ingredients Date

10/15/01

Lee B. Dexter, Technical Consultant

Section III
Information on Self-Limiting Levels of Use and Probable Consumption

A. Self-Limiting Levels of Use

While there are few published studies on the self-limiting levels of chitosan use in foods, several factors are expected to limit the use of the ingredient in food formulations. First, chitosan is a very expensive ingredient, with a value in excess of US $20 per pound. Second, studies by food scientists have shown that chitosan has been an effective emulsifier at percentages of use of less than 1.0% [Poole, 1988 Vol 11 Tab 225 and Rodriguez, *et al.*, 2000 Vol 12 Tab 237].

Excessive chitosan usage may produce aggregation of ingredients, depending on the synergies involved and the pH of the formulation [Poole, 1988 Vol 11 Tab 225]. Poole found that use of greater than 0.8% chitosan yielded a dense aggregate in a whey protein isolate-corn oil emulsion [Poole, 1988 Vol 11 Tab 225]. Further, Rodriguez *et al.*, 2000, stated that the affinity between chitosan and water decreased with increasing pH [Rodriguez, *et al.*, 2000 Vol 12 Tab 237].

Therefore, use of chitosan is expected to be limited by its physicochemical properties. Rodriguez *et al.*, 2000, concluded that the behavior of chitosan in systems containing different chitosan/oil ratios was due to certain properties of chitosan. The authors found that the surface tension of chitosan solutions (1% v/v acetic acid aqueous solution) was higher than that of the solvent (water). This implied that chitosan is strongly excluded from the air/solution interface. According to the researchers, the phenomena are consistent with expectations derived from the theoretical behaviors of the cationic $-NH_3^+$ and alcoholic –OH groups distributed on the molecules structure [Rodriguez *et al.*, 2000 Vol 12 Tab 237].

Primex submits that food processors will be involved in a great deal of applications development, in order to determine that most economical uses for chitosan.

AM

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MEMORANDUM

TO: KATHLEEN MCAVENEY JONES

FROM: LEE B. DEXTER

DATE: NOVEMBER 5, 2001

RE: 8 MISSING PAGES TO GRN: 74 AMENDMENT

Dear Dr. McAveney Jones:

Please find attached the missing 8 pages from the GRN: 74 Amendment. One item, I was unsure about was the issue of meats. Given USDA's comments, I have left the potential intake for fresh meats, meat products, and fresh poultry, and poultry products at 0. I have included fish and fish products at levels consistent with the use of chitosan films. If this is not satisfactory, please let me know.

Several papers have now been published on the use of chitosan film as an antimicrobial for meats. Perhaps at some point in the future, Primex will want to submit a Notice expanding the uses into those categories.

Best regards

B. Probable Consumption

A Realistic Estimate of Exposure to Shrimp-Derived Chitosan

Primex is providing a realistic estimate of exposure to Shrimp-Derived Chitosan. The FDA's guidance document "*Estimating Exposure to Direct Food Additives and Chemical Contaminants in the Diet*", USDA's *Continuing Survey of Food Intakes by Individuals* (CSFII, 1996), and recent market surveys were used as references to estimate the realistic exposure to Shrimp-Derived Chitosan produced by Primex [Enns, *et al.*, 1997 Vol 4 Tab 36].

1. Exposure Estimate

In order to estimate the probable human exposure to Shrimp-Derived Chitosan on a continual daily basis, Primex has relied upon examples of commercial formulations from Asia and Europe, examples of potential Shrimp-Derived Chitosan use from recent published studies, the patent literature, and the results of its own food technology research. Exposure to Shrimp-Derived Chitosan was estimated in accordance with the agency's guidance document, cited above, "*Estimating Exposure to Direct Food Additives and Chemical Contaminants in the Diet*" [FDA, September 1995]. Use of the material was estimated for the 94 NAS food categories, on a working Table titled "GRAS Food Additive Categories and Sub-Categories" (data not shown). Use levels as a percent of the final food products were then estimated across food sub-categories to yield averages for the major food groups. For instance, all of the 16 sub-categories in the Baked Goods category were averaged to yield a "Baked Goods" use level that was entered into Table 1. Table 1 lists the 43 Food Categories at 21 CFR §170.3 (n). Since neither the "GRAS Food Additive Categories and Sub-categories", nor the "43 Food Categories" include food intake data, the average use levels for the sub-food categories shown on Table 1 were then re-averaged, if necessary, so that they were consistent with the food categories from USDA's *Continuing Survey of Food Intakes by Individuals* (CSFII) [Enns, *et al.*, 1997 Vol 2 Tab 11]. The CSFII food categories, along with Shrimp-Derived Chitosan use level, food sub-category, mean intake, and Shrimp-Derived Chitosan intake are shown in Table 2. This Table presents mean intake data for adults 20 years and over (eaters only).

For example, three food sub-categories (White Bread and Rolls, Dark Bread and Rolls, and Bread, Other) from the NAS list were averaged to yield the average use level for Yeast Bread and Rolls shown on Table 2 (the CSFII categories). Since the CSFII intake data is statistically valid, percentage use levels for Shrimp-Derived Chitosan may be multiplied by the mean intakes indicated in the CSFII database to yield an estimated exposure to Shrimp-Derived Chitosan from a given food category. The resulting totals for each food category were then summed to yield a total mean daily intake.

Note that zeros were entered for Shrimp-Derived Chitosan for certain food categories where Shrimp-Derived Chitosan use is unlikely, e.g., in coffee and teas. The total daily mean intake from Table 2 was doubled to estimate the 90th percentile (in accordance with the guidance document).

The results of these calculations and potential exposure from other sources are summarized at the bottom of Table 2. The estimated daily mean exposure derived from Table 2 was 1.49 grams of Shrimp-Derived Chitosan per day. The 90th percentile is then estimated to be 2.98 grams per day. Contributing to these totals were 0.267 grams per day from bakery goods, 0.25 grams per day from grain products, 0.081 grams per day from fruit products, and 0.339 grams per day from milk products.

Primex Ingredients is also aware that certain individuals may experience additional exposure to Shrimp-Derived Chitosan from its use as a source of dietary fiber in dietary supplement form [Enns, *et al.*, 1997 and Langhelle, 1999 Vol 8 Tab 126]. The recommended dose of chitosan as a dietary supplement is 1.5 to 3.0 grams per day. This dosage has been recommended based on the results of clinical trials with human volunteers [Colombo, *et al.*, 1996, Vol 3 Tab 24].

While the percentage of use assigned to each food subcategory was considered realistic, this exposure estimate remains conservative because 100% market penetration was assumed, and Shrimp-Derived Chitosan was assumed to be included in all products in a given subcategory. Additionally, the values presented below assume that 100% of consumers are "eaters" of chitosan-containing products within a given food category.

In reality, chitosan is expected to be used in only certain products within a given subcategory. Further, use within a subcategory will be

limited by economics and organoleptic considerations. Market development in the US is likely to be directed towards the functional properties of chitosan, and certain functional properties may not be appropriate for a given consumer product. An example of this is that chitosan tends to increase viscosity in batter or fluid food systems. This may not create a desirable product in all cases.

Additionally, Primex Shrimp-Derived Chitosan is a very expensive food ingredient, costing US$ 20-30 dollars per kilogram. Therefore, those applications in which it will be cost effective will need to be carefully chosen, and will most likely dictate that chitosan will be used at the minimum level necessary to produce a given technical or nutritional effect.

Table 1 below shows the realistic percentages of use assigned to each of the forty-three food categories listed at 21 CFR § 170.3 (n). The correlation between the major food categories derived from the USDA food survey, and those listed at § 170.3 (n) is shown in Table 3.

The mean and the 90th percentile of estimated exposure to Primex Shrimp-Derived Chitosan remains lower than the recommended levels of intake for dietary fiber (25-30 grams per day) in the United States [Enns, *et al.,* 1997 Vol 4 Tab 36]. Additionally, dietary fiber intake in the US averaged for men and women has been estimated by USDA at 16.1 grams per day [Enns, *et al.,* 1997 Vol 4 Tab 36]. This value still represents a deficit in relation to levels established for good health. It is possible that consumption of chitosan may provide health benefits from additional dietary fiber intake. Further, no untoward effects have been reported in multiple studies involving chitosan consumption by human volunteers consuming similar amounts of chitosan [See Section IV, Safety]. Therefore, Primex submits that the exposure to chitosan represented by this estimate will pose no threat to the public health.

Section III:
Information on Self-Limiting Levels of Use and Probable Consumption

Table 1
Use of Shrimp-Derived Chitosan In the Food Categories Listed by the US FDA At 21 CFR §170.3 (n)

	Food Categories	Realistic Percentage of Use in the US Market
01	Baked Goods, Baking Mixes	0.035%
02	Beverages alcoholic	0%
03	Beverages and beverage bases, non-alcoholic	0.625%
04	Breakfast Cereals	0.032%
05	Cheeses	0.56%
06	Chewing Gum	0%
07	Coffee and tea	0%
08	Condiments and relishes	0.3%
09	Confections and frostings	0
10	Dairy product analogs	0.56%
11	Egg Products	0.213%
12	Fats and oils	0.22%
13	Fish Products	0.022%
14	Fresh eggs	0%
15	Fresh fish	0.022%
16	Fresh fruits and fruit juices	0.46%
17	Fresh Meats	0%
18	Fresh poultry	0%
19	Fresh vegetables	0.21%
20	Frozen dairy desserts and mixes	0.77%
21	Fruit and water ices	0%
22	Gelatins, puddings and fillings	0%
23	Grain products and pastas	0.026%
24	Gravies and Sauces	0%
25	Hard candy	0%
26	Herbs, seeds, spices, seasonings, blends	0%
27	Jams and jellies, home prepared	0%
28	Jams and jellies commercial	0.25%
29	Meat products	0%
30	Milk, whole and skim	2.0%
31	Milk products	0.77%
32	Nuts and Nut products	0%
33	Plant protein products	2.2%
34	Poultry products	0%
35	Processed fruits and fruit juices	0.46%
36	Processed vegetables and vegetable juices	0.21%
37	Snack foods	0.75%
38	Soft Candy	0.5%
39	Soups, home prepared	0%
40	Soups and soup mixes	0.85%
41	Sugar, white granulated	0%
42	Sugar substitutes	0%
43	Sweet Sauces, toppings, and syrups	0%

Table 2
Estimated Intake of Shrimp-Derived Chitosan in Selected Food Categories

Major Food Codes	Food Category	Chitosan Solids Use Level (%)	Food Intake (g)	Chitosan Solids Intake (g)	CSFII
	Baked goods, baking mixes				
51	Yeast Breads and Rolls	0.0256	50	0.0128	1996
52, 55	Quick breads, pancakes, etc.	0.04	20	0.1	1996
53	Cakes, cookies, pastries, pies[1]	0.04	38	0.0152	1996
58	Mixtures mainly grain	0.153	107	0.139	1996
	Breakfast cereals			0.287	
571 - 574, 578	Ready-to-eat cereals	0.032	17	0.085	1996
	Grain products and pastas				
562	Rice	0.0256	19	0.076	1996
561	Pasta	0.0256	21	0.084	1996
54	Snack foods(crackers, chips)	0.75	12	0.09	1996
	Total Vegetables		132		1996
	Fresh vegetables [2]	0.21	45	0.0945	1996
	Processed vegetables, juices	0.21	14	0.0294	1996
	Dark Green Vegetables	0.21	13	0.0273	
	Total Fruits				1996
612	Citrus juices [3]	0.46	17.7	0.0814	1996
621	Dried fruits	0	1	0	1996
641,642	Non-citrus juices and nectars	0	26	0	1996
	Fruits and mixtures	0	0	0	1996
	Milk products				
114	Yogurt	0.77	8	0.062	1996
131,132, 133, 134	Milk desserts [4]	0.77	24	0.096	1996
140-147	Cheese	0.56	8	0.0448	1996
116, 118	Milk-Based Beverages[5]	0.2	34	0.136	1968
	Meat Products				
252	Sausages, processed meats	0	21	0	1996
	Pork	0	5	0	1996
	Fish	0.022	11	0.0024	
	Eggs				
321,323,324, 33,34	Egg Products[6]	0.213	9	0.0192	1996
	Legumes				
	Legumes[7]	2.2	1.8	0.0396	1996
	Nuts and Nut Products				
	Nuts and Nut Products	0	3	0	1996
	Fats and Oils				
832	Salad dressings	0.22	8	0.00176	1996
	Total sugars and sweets				
917,918	Candy (Soft and Hard)	0.5	7	0.035	1996
911 - 916	Other Sugar Products[8]	0.25	15	0.0375	1996
	Beverages Non-Alcoholic				
	Coffee	0	254	0	1996
923	Teas	0	128	0	1996
925	Low Cal.Fruit drinks and ades	1	18	0.18	1996
92400100	Low Cal. Carbonated drinks	0	74	0	1996
	Total Mean Intake (grams)			**1.7559**	
	90th Percentile			**2.9778**	
	Potential Dietary Supplement Use			**1.5-3.0**	

1) Includes dietetic bars
2) Includes tomatoes and lettuce products
3) Includes citrus juices with pulp
4) Includes low fat ice creams etc.
5) Includes only skim milk products
6) Includes processed egg products
7) Includes only Tofu and similar products
8) Includes Jams, Jellies, etc.

Section III:
Information on Self-Limiting Levels of Use and Probable Consumption

Table 3
USDA Major Food Categories And Corresponding Categories From 21 CFR 170.3 (n)

USDA Food Category[1]	Categories Listed In 170.3 (n)[2]
Total Grain Products	01, 04, 23, 37
Dietary Supplement; Source of Soluble Fiber	
Vegetables and Vegetable Dishes	36
Fruits and Fruit Preparations	16, 35
Milk Desserts/Cheese Products	5, 20, 22, 31,10
Fish and Fish Products	13, 15
Total Fats and Oils	12
Legumes and Legume Dishes	33, 36
Sugars and Sweets	06, 09, 25, 28, 38, 42, 43
Nuts and Seeds	32
Total Non-Alcoholic Beverages (excluding coffee and fruit juices)	16, 35, 03

1. Major food categories are those used in USDA's Continuing Survey of Food Intake by Individuals [Enns, *et al.*, 1997 Vol 4 Tab 36].
2. Column 2 contains the number assigned to the various food categories as listed in 21 CFR § 170.3 (n), which are contained within those reported by Enns, *et al.*, 1997 [Enns, *et al.*, 1997 Vol 4 Tab 36].

000274

2. Recently Developed Applications

One of the most cost effective uses of Shrimp-Derived Chitosan identified by Primex, is its use as an antimicrobial to retard spoilage in various foods (Rhoades, et al., 2000, *Applied and Environmental Microbiology* (66): No 1). Rhoades, et al., 2000 reported that the addition of 0.3g/L of chitosan (either native or degraded), when added to refrigerated items with short shelf lives, such as chickpea hummus or pasteurized apple-elderberry juice was able to reduce the microbiological content of these products by approximately 3 to 4 log CFU/ml. Yeast levels were completely reduced by the addition of chitosan to the products, and remained below the level of detection even after several days of storage.

Several research groups have published results on the use of chitosan to produce antimicrobial films (Begin, et al., 1999, *International Journal of Biological Macromolecules*: (1):63-67; Nunthanid, et al., 2001, *Drug Development and Industrial Pharmacology* (2):143-57; Ouattar, et al., 2000, *International Journal of Food Microbiology*: 62(1-2):139-48; Tsai, et al., 1999, *Journal of Food Protection* 62(3):239-243; and Roller, et al., 2000, *Journal of Food Protection* 63(2):202-9. The films contained either chitosan alone or chitosan in combination with other antimicrobials, such as propionic acid. Low levels of chitosan solids were used to produce the films (0.5% to 2.0%) in acetic or other food grade acids.

These applications trials by the authors cited above, as well as others indicated that low levels of chitosan solids either as a film or a powder could be used to increase the shelf life of foods susceptible to microbial degradation. For instance, the investigation by Roller, et al., 2000 showed that 3g/L of chitosan in combination with 0.16% acetic acid slowed growth of *Lactobacillus fructivorans*, and that a coating of chitosan (9mg/g of shrimp) reduced the growth of spoilage microorganisms from 8 CFU/g to 4 CFU/g in shrimp salad, when stored at 5°C. Tsai, et al., 1999 suggested that the antibacterial mechanism of shrimp chitosan may be due to a cross-linkage between the polycations of chitosan, and the anions on the surface of bacteria, such as *E. coli*, thus causing leakage of glucose and lactate dehydrogenase from the cell. Primex is aware that other foods, such as fresh fruits and vegetables may become candidates for chitosan film coverings to retard spoilage and preserve appearance. Based on the expanding knowledge base surrounding these types of applications for Shrimp-Derived Chitosan, as well as its recent market survey, Primex believes that the mean consumption of chitosan will not exceed 1.49 grams per day in a normal diet.

Section III:
Information on Self-Limiting Levels of Use and Probable Consumption

Recently Identified Applications References

1. Begin, *et al.*, 1999, Antimicrobial Films Produced from Chitosan. *International Journal of Biological Macromolecules*: (1):63-67.

2. Nunthanid, *et al.*, 2001, Physical Properties and Molecular Behavior of Chitosan Films. *Drug Development and Industrial Pharmacology* (2):143-57.

3. Ouattar, *et al.*, 2000, Inhibition of Surface Spoilage Bacteria in Processed Meats by Application of Antimicrobial Films Prepared with Chitosan. *International Journal of Food Microbiology*. 62(1-2):139-48.

4. Roller, *et al.*, 2000, The Antimicrobial Properties of Chitosan in Mayonnaise and Mayonnaise-Based Shrimp Salads. Journal *of Food Protection* 63(2):202-9.

5. Rhoades, *et al.*, 2000, Antimicrobial Actions of Degraded and Native Chitosan Against Spoilage Organisms in Laboratory Media and Foods. *Applied and Environmental Microbiology*. 66: (1): 80-86.

6. Tsai, *et al.*, 1999, Antibacterial Activity of Shrimp Chitosan Against *Eschericia coli*. *Journal of Food Protection* 62(3):239-243

AM

LEE B. DEXTER & ASSOC.
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November 30, 2001

Via Fax: Pages 4

Dr. Kathleen McAveney Jones
U.S. Food and Drug Administration
Center for Food, Safety and Nutrition
Office of Food Additive Safety
Division of Biotech and GRAS Notice Review, HFS 206
200 C St. SW
Washington, DC 20204

Re: GRAS Notice 74: Shrimp-Derived Chitosan

Dear Dr. McAveney Jones:

In a letter dated October 26, 2001 Primex Ingredients (Primex) of Avaldsnes, Norway amended its notice of a claim that the use of shrimp-derived chitosan as a food ingredient is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act, and provided a revised estimate of mean consumption levels for shrimp-derived chitosan. In revising its consumption estimate, Primex also provided revised Tables 1 and 2 (pages 5 and 6) of Section III of its Notification. These tables showed that Primex did not include any use of shrimp-derived chitosan in red meat or poultry products. At this time, the company would like to specifically state that it does not intend to include uses for shrimp-derived chitosan in red meats and poultry in its GRAS claim. Rather, the company will resubmit a GRAS Notification for the intended use of shrimp-derived chitosan in red meats and poultry at a later time, after sufficient data and information have been gathered to substantiate the technical effect of shrimp-derived chitosan in meat and meat products.

Also in this letter Primex would like to point out a typographical error in Table 1 (mentioned above). This Table should have shown an estimated use level for shrimp-derived chitosan in "Milk, whole and skim" (Food Category # 30 of 21 CFR §170.3(n)) as 0.2%, not 2.0%. This value will then correspond to the Milk-Based Beverages category shown on Table 2 (also mentioned above). Both Tables are being submitted with this letter for clarification.

Primex would like to thank the agency for the opportunity to amend and correct its submission, and the company appreciates the agency's patience with this correction. I will be happy to respond to any further technical questions regarding this submission, and my contact information is shown on the letterhead.

Again, thank you for the opportunity to correct GRAS Notice 74. Attached to this letter are Tables 1 and 2 of Section III in GRAS Notice 74.

Sincerely,

Lee B. Dexter
Technical Consultant

CC: Dr. Linda Kahl, CFSAN
Mr. Steve Dillingham, Norwegian Trade Council
Mr. Bjarte Langhelle, Primex
Mr. Thore Ivar Thorsen, Primex

Section III:
Information on Self-Limiting Levels of Use and Probable Consumption

Table 1
Use of Shrimp-Derived Chitosan In the Food Categories Listed by the US FDA At 21 CFR §170.3 (n)

	Food Categories	Realistic Percentage of Use in the US Market
01	Baked Goods, Baking Mixes	0.035%
02	Beverages alcoholic	0%
03	Beverages and beverage bases, non-alcoholic	0.625%
04	Breakfast Cereals	0.032%
05	Cheeses	0.56%
06	Chewing Gum	0%
07	Coffee and tea	0%
08	Condiments and relishes	0.3%
09	Confections and frostings	0
10	Dairy product analogs	0.56%
11	Egg Products	0.213%
12	Fats and oils	0.22%
13	Fish Products	0.022%
14	Fresh eggs	0%
15	Fresh fish	0.022%
16	Fresh fruits and fruit juices	0.46%
17	Fresh Meats	0%
18	Fresh poultry	0%
19	Fresh vegetables	0.21%
20	Frozen dairy desserts and mixes	0.77%
21	Fruit and water ices	0%
22	Gelatins, puddings and fillings	0%
23	Grain products and pastas	0.026%
24	Gravies and Sauces	0%
25	Hard candy	0%
26	Herbs, seeds, spices, seasonings, blends	0%
27	Jams and jellies, home prepared	0%
28	Jams and jellies commercial	0.25%
29	Meat products	0%
30	Milk, whole and skim	0.2%
31	Milk products	0.77%
32	Nuts and Nut products	0%
33	Plant protein products	2.2%
34	Poultry products	0%
35	Processed fruits and fruit juices	0.46%
36	Processed vegetables and vegetable juices	0.21%
37	Snack foods	0.75%
38	Soft Candy	0.5%
39	Soups, home prepared	0%
40	Soups and soup mixes	0.85%
41	Sugar, white granulated	0%
42	Sugar substitutes	0%
43	Sweet Sauces, toppings, and syrups	0%

Table 2
Estimated Intake of Shrimp-Derived Chitosan in Selected Food Categories

Major Food Codes	Food Category	Chitosan Solids Use Level (%)	Food Intake (g)	Chitosan Solids Intake (g)	CSFII
	Baked goods, baking mixes				
51	Yeast Breads and Rolls	0.0256	50	0.0128	1996
52, 55	Quick breads, pancakes, etc.	0.04	20	0.1	1996
53	Cakes, cookies, pastries, pies[1]	0.04	38	0.0152	1996
58	Mixtures mainly grain	0.153	107	0.139	1996
	Breakfast cereals			0.267	
571 - 574, 578	Ready-to-eat cereals	0.032	17	0.085	1996
	Grain products and pastas				
562	Rice	0.0256	19	0.076	1996
561	Pasta	0.0256	21	0.084	1996
54	Snack foods(crackers, chips)	0.75	12	0.09	1996
	Total Vegetables		132		1996
	Fresh vegetables [2]	0.21	45	0.0945	1996
	Processed vegetables, juices	0.21	14	0.0294	1996
	Dark Green Vegetables	0.21	13	0.0273	
	Total Fruits				1996
612	Citrus juices [3]	0.46	17.7	0.0814	1996
621	Dried fruits	0	1	0	1996
641,642	Non-citrus juices and nectars	0	26	0	1996
	Fruits and mixtures	0	0	0	1996
	Milk products				
114	Yogurt	0.77	8	0.062	1996
131,132, 133, 134	Milk desserts [4]	0.77	24	0.096	1996
140-147	Cheese	0.56	8	0.0448	1996
116, 118	Milk-Based Beverages[5]	0.2	34	0.136	1968
	Meat Products				
252	Sausages, processed meats	0	21	0	1996
	Pork	0	5	0	1996
	Fish	0.022	11	0.0024	
	Eggs				
321,323,324, 33,34	Egg Products[6]	0.213	9	0.0192	1996
	Legumes				
	Legumes[7]	2.2	1.8	0.0396	1996
	Nuts and Nut Products				
	Nuts and Nut Products	0	3	0	1996
	Fats and Oils				
832	Salad dressings	0.22	8	0.00176	1996
	Total sugars and sweets				
917,918	Candy (Soft and Hard)	0.5	7	0.035	1996
911 - 916	Other Sugar Products[8]	0.25	15	0.0375	1996
	Beverages Non-Alcoholic				
	Coffee	0	254	0	1996
923	Teas	0	128	0	1996
925	Low Cal.Fruit drinks and ades	1	18	0.18	1996
92400100	Low Cal. Carbonated drinks	0	74	0	1996
	Total Mean Intake (grams)			1.7559	
	90th Percentile			2.9778	
	Potential Dietary Supplement Use			1.5-3.0	

1) Includes dietetic bars
2) Includes tomatoes and lettuce products
3) Includes citrus juices with pulp
4) Includes low fat ice creams etc.
5) Includes only skim milk products
6) Includes processed egg products
7) Includes only Tofu and similar products
8) Includes Jams, Jellies, etc.

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TO: DR. KATHLEEN MCAVENEY JONES, CFSAN
VIA FAX: (202) 418-3131

FROM: LEE B. DEXTER

PAGES: 2

DATE: JANUARY 31, 2002

RE: LETTER WITHDRAWING GRAS NOTIFICATION NUMBER 000073

Dear Dr. Jones:

Please find attached a letter withdrawing the GRAS Notification for shrimp-derived chitosan. Primex Ingredients, ASA would like to withdraw without prejudice. The company would like to schedule a meeting with appropriate CFSAN personnel, so that Primex can determine if recently published and unpublished studies are sufficient to resolve any of the concerns the agency may have concerning the safety of this material, prior to submitting a new and revised document.

Please accept my thanks for calling me in advance on this issue.

LEE B. DEXTER & ASSOC.
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January 29, 2002

Dr. Kathleen McAveney Jones
Office of Food Additive Safety
Center for Food, Safety and Nutrition
Food and Drug Administration
200 C St. SW HFS-206
Washington, DC 20204

Dear Dr. Jones:

Primex Ingredients ASA GRAS Notification for Shrimp-Derived Chitosan
GRN No. 000073

Primex Ingredients, ASA, Avaldsnes, Norway hereby notifies the Center for Food Safety and Applied Nutrition (CFSAN), Office of Food Additive Safety, that the company withdraws its GRAS Notification for shrimp-derived chitosan (GRN 000073) without prejudice. It is the intention of Primex Ingredients ASA to resubmit a revised notice of a claim for premarket exemption based on a GRAS determination under proposed §170.36, following discussions with agency personnel.

In order to determine whether newly published and unpublished studies may assist in creating a consensus around the safety of shrimp-derived chitosan, the company would like to schedule a meeting to discuss GRN 000073. Following the meeting, the company will prepare a revised Notification, which will incorporate any pertinent information contained in recent publications.

Primex Ingredients ASA wishes to thank CFSAN officials for their cooperation and attention in reviewing GRN 000073. On the company's behalf, I will telephone to make arrangements for a meeting time convenient to both the agency and Primex.

Sincerely.

Lee B. Dexter
Technical Consultant

CC: Bjarte Langhelle, Primex Ingredients ASA
Thore Ivar Thorsen, Primex Ingredients ASA
Steven Dillingham, Norwegian Trade Council

Reference List for Industry Submission, GRN 000073

Pages	*Author*	*Title*	*Publish Date*	*Publisher*	*BIB_Info*
000281 - 000291	Koide, S. S.	Chitin-Chitosan: Properties, Benefits And Risks	1998	Nutrition Research	Volume 18, Number 6, pgs 1091-1101
000292 - 000294	Pittler, M. H.; Abbot, N. C.; Harkness, E. F.; Ernst, E.	Randomized, double-blind trial of chitosan for body weight reduction	1999	European Journal of Clinical Nutrition	Volume 53, pgs 379-381
000295 - 000300	Deuchi, Keiji; Kanauchi, Osamu; Shizukuishi, Mika; Kobayashi, Eiichi	Continuous and Massive Intake of Chitosan Affects Mineral and Fat-soluble Vitamin Status in Rats Fed on a High-fat Diet	1995	Biosci. Biotech. Biochem.	Volume 59, Number 7, pgs 1211-1216
000301 - 000303	Wada, Masahiro; Nishimura, Yoshikazu; Watanabe, Yoshito; Takita, Toshichika; Innami, Satoshi	Accelerating Effect of Chitosan Intake on Urinary Calcium Excretion by Rats	1997	Biosci. Biotech. Biochem.	Volume 61, Number 7, pgs 1206-1208
000304 - 000319	Sciutto, Colombo P.	Nutritional aspects of chitosan employment in hypocaloric diet	1996	Acta Toxicol. Ther.	Volume XVII, Number 4, pgs 287-302

NA- Not applicable