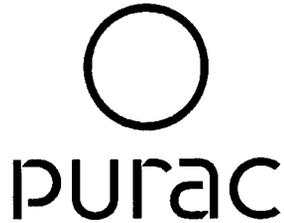
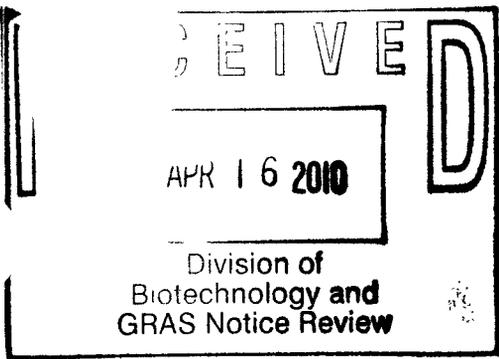


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ORIGINAL SUBMISSION

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Robert L. Martin, Ph.D.
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food And Drug Administration
5100 Paint Branch Parkway
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Gorinchem, April 12, 2010

CONFIDENTIAL

Re: GRAS Notice for ϵ -Polylysine

Dear Dr. Martin,

In accordance with 21 CFR §170.36 [Notice of a claim for exemption based on a Generally Recognized As Safe (GRAS) determination] published in the Federal Register [62 FR 18938 (17 April 1997)], I am submitting in triplicate, as the Notifier [Purac Blochem b.v., Arkelsedijk 46 PO Box 21, 4206 AA Gorinchem, The Netherlands], a Notice of the determination, on the basis of scientific procedures, that ϵ -polylysine distributed by Purac, as defined in the enclosed documents, is GRAS under specific conditions of use as a preservative in various traditional foods, and therefore, is exempt from the premarket approval requirements of the Federal, Food, Drug and Cosmetic Act. Information setting forth the basis for the GRAS determination, includes a comprehensive summary of the data available that has been reviewed by an independent panel of experts (the Expert Panel) qualified by scientific training and experience to evaluate the safety of ϵ -polylysine in traditional food products. A fourth copy of this Notification also has been included for purview by the United States Department of Agriculture's Food Safety and Inspection Service regarding the uses of ϵ -polylysine as a preservative in meat and poultry, and meat and poultry containing products.

Should you have any questions or concerns regarding this GRAS Notice, please do not hesitate to contact me at any point during the review process so that we may provide a response in a timely manner.

Sincerely,

(b) (6)

Ton van Dongen
Global Regulatory Manager
Purac Blochem b.v.

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EPSILON-POLYLYSINE GRAS NOTICE

Prepared for: Robert L. Martin, Ph.D.
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Prepared by: Purac Biochem b.v.
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The Netherlands

April 05, 2010

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EPSILON-POLYLYSINE GRAS NOTICE

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I GRAS EXEMPTION CLAIM

A. Claim of Exemption From the Requirement for Premarket Approval Pursuant to Proposed 21 CFR §170.36(c)(1) [62 FR 18938 (17 April 1997)] (U.S. FDA, 1997)

As defined herein, epsilon-polylysine (ϵ -polylysine) produced *via* a fermentation process using *Streptomyces albulus* subsp. *lysinopolymerus*, has been determined by Purac Biochem b.v. (Purac) to be Generally Recognized as Safe (GRAS) for use as a preservative in various food types described herein, consistent with Section 201(s) of the *Federal Food, Drug, and Cosmetic Act* (U.S. FDA, 2009a). This determination is based on scientific procedures as described in the following sections, and on the consensus opinion of an independent panel of experts qualified by scientific training and expertise to evaluate the safety of ϵ -polylysine under the conditions of intended use in food. Therefore, the use of ϵ -polylysine in food as described below is exempt from the requirement of premarket approval (Section 409 of the *Federal Food, Drug and Cosmetic Act*) (U.S. FDA, 2009b).

Signed,

(b) (6)

Ton van Dongen
Global Regulatory Manager
Purac Biochem b.v.
t.van.dongen@purac.com

Date April 12, 2010

B. Name and Address of Notifier

Purac Biochem b.v.
Arkelsedijk 46
PO Box 21
4206 AA Gorinchem
The Netherlands
+31 183 695 730



C. Common Name of the Notified Substance

Epsilon-polylysine; ϵ -polylysine; polylysine

D. Conditions of Intended Use in Food

Purac intends to market ϵ -polylysine produced *via* a fermentation process using *Streptomyces albulus* subsp. *lysinopolymerus*, as a food ingredient in the United States for use as a preservative in the proposed food categories as described in Table A-1 (Appendix A), at levels ranging from 0.005 to 0.06%. These uses include applications in meat and poultry, and meat and poultry containing products.

E. Basis for the GRAS Determination

Pursuant to 21 CFR §170.30(b), ϵ -polylysine has been determined by Purac to be GRAS on the basis of scientific procedures (U.S. FDA, 2009c). This GRAS determination is based on data generally available in the public domain pertaining to the safety of ϵ -polylysine for use in food, as discussed herein and in the accompanying documents, and on consensus among a panel of Experts¹ (The Panel) who are qualified by scientific training and experience to evaluate the safety of ϵ -polylysine as a component of food.

F. Availability of Information

Data and information that serve as the basis for this GRAS Notice will be sent to the U.S. Food and Drug Administration (FDA) upon request, or will be available for review and copying at reasonable times at the offices of Purac located at the following address:

Purac Biochem b.v.
Arkelsedijk 46
PO Box 21
4206 AA Gorinchem
The Netherlands

Should the FDA have any questions or additional information requests regarding this Notice, Purac also will supply these data and information.

¹ The Panel consisted of: Dr. Ian C. Munro, Ph.D. (Cantox Health Sciences International), Dr. Stanley M. Tarka, Ph.D. (The Tarka Group, Inc.), and Dr. John Thomas, Ph.D. (Indiana University School of Medicine). These Panel members are considered qualified, through their relevant experience and scientific training, to evaluate the safety of ϵ -polylysine under the proposed food uses.

II. DETAILED INFORMATION REGARDING THE IDENTITY OF THE SUBSTANCE

A. Identity

(i) Common or Usual Name

Epsilon-Polylysine; ϵ -Polylysine; Polylysine

(ii) Chemical Name

poly(imino(2-amino-1-oxo-1,6-hexanediyl)

(iii) Trade Name

Polylysine will be sold under the PuraQ trade name. Currently the products available are:

PuraQ Xtend FX25 (25% solution in water)

PuraQ Xtend FX50P (50:50 mixture with maltodextrin).

Other products containing polylysine as an ingredient may be developed, respecting the proposed food uses and corresponding use levels.

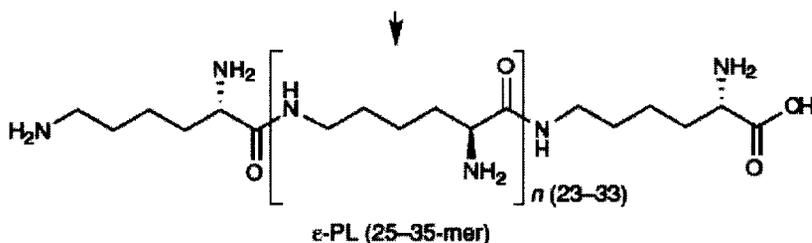
(iv) Chemical Abstract Service (CAS) Number

28211-04-3

(v) Chemical and Physical Characteristics

Epsilon-Polylysine is a homopolymer of L-lysine, one of the essential amino acids. The chemical formula for ϵ -polylysine is shown in Figure II.A-1. The empirical formula for the typical ϵ -polylysine homopolymer is $C_{180}H_{362}N_{60}O_{31}$ with a molecular weight of approximately 4700 Da.

Figure II.A-1 ϵ -Polylysine



B. Method of Manufacture

(i) History

More than 20 years ago the Gram-positive bacterium *Streptomyces albulus* subsp. lysinopolymerus strain 346 was isolated from Japanese soil, and observed to display broad spectrum antimicrobial activity. During fermentation, the strain was shown to be capable of synthesizing and excreting polylysine at high concentrations of up to 5 g/L (Shima and Sakai, 1977). Later a mutant of strain 346 was isolated which produced 4 times higher amounts of ϵ -polylysine (Hiraki *et al.*, 1998).

Streptomyces albulus is classified as a nonpathogenic microorganism of the Order *Actinomycetales* and Family *Streptomycetaceae*. First isolated in 1977 from soil, *Streptomyces albulus* is used exclusively for the commercial production of ϵ -polylysine, and it is one of the few organisms that have been found to produce the compound in significant quantities (Yoshida and Nagasawa, 2003). Various *Streptomyces* species have a long-history of use in the production of food ingredients. For example, *Streptomyces griseus* (21 CFR §184.1945) is considered GRAS for use in cyanocobalamin production; and *Streptomyces rubinoginosis*, *Streptomyces olivaceus*, and *Streptomyces olivochromogenes* (21 CFR §184.1372) are GRAS affirmed sources of insoluble glucose isomerase enzymes for use in food production (U.S. FDA, 2009c).

(ii) Manufacturing

ϵ -Polylysine is manufactured using an aerobic fermentation process using the non-toxicogenic non-pathogenic microorganism *Streptomyces albulus* subsp. lysinopolymerus. All raw materials and processing aids used in the manufacture of ϵ -polylysine are suitable food-grade materials and are used in accordance with applicable U.S. federal regulations as described in Table II.B-1 below. An overview of the manufacturing process is presented in Figure II.B-1 below. The biotechnological process for producing ϵ -polylysine is described under the U.S. Patent Number 5,900,363 (PN 5,900,363). The fermentation is conducted according to recognized principles of current Good Manufacturing Practices (cGMP) using common and suitable food grade raw materials². Following fermentation, the microorganism is removed from the culture media *via* a filter sterilization step (0.1 μ m membrane filter), and the filtrate is then purified using chromatographic separation³. Three ion-exchange resins are used sequentially in the purification process: a weak acid, carboxyl cationic-exchange resin (Amberlite IRC-50, Rohm & Hass, eluent: 0.2M NaOH); a strong base, -N(CH₃)₃ anionic-exchange resin [Amberlite IRA-402, Rohm and Hass, eluent: water], and a strong acid (403) cationic-exchange resin (Amberlite XT

² Fermentation media contains the following: potassium dihydrogen phosphate, dipotassium hydrogen phosphate, yeast extract, ammonium sulfate, magnesium sulfate, zinc sulfate, ferrous sulfate, glucose, sodium hydroxide, and ammonia.

³ Microbial analyses of the filter purified ϵ -polylysine solution prior to spray drying, confirms that the filter sterilization step has effectively removed the fermentation microbe from solution.

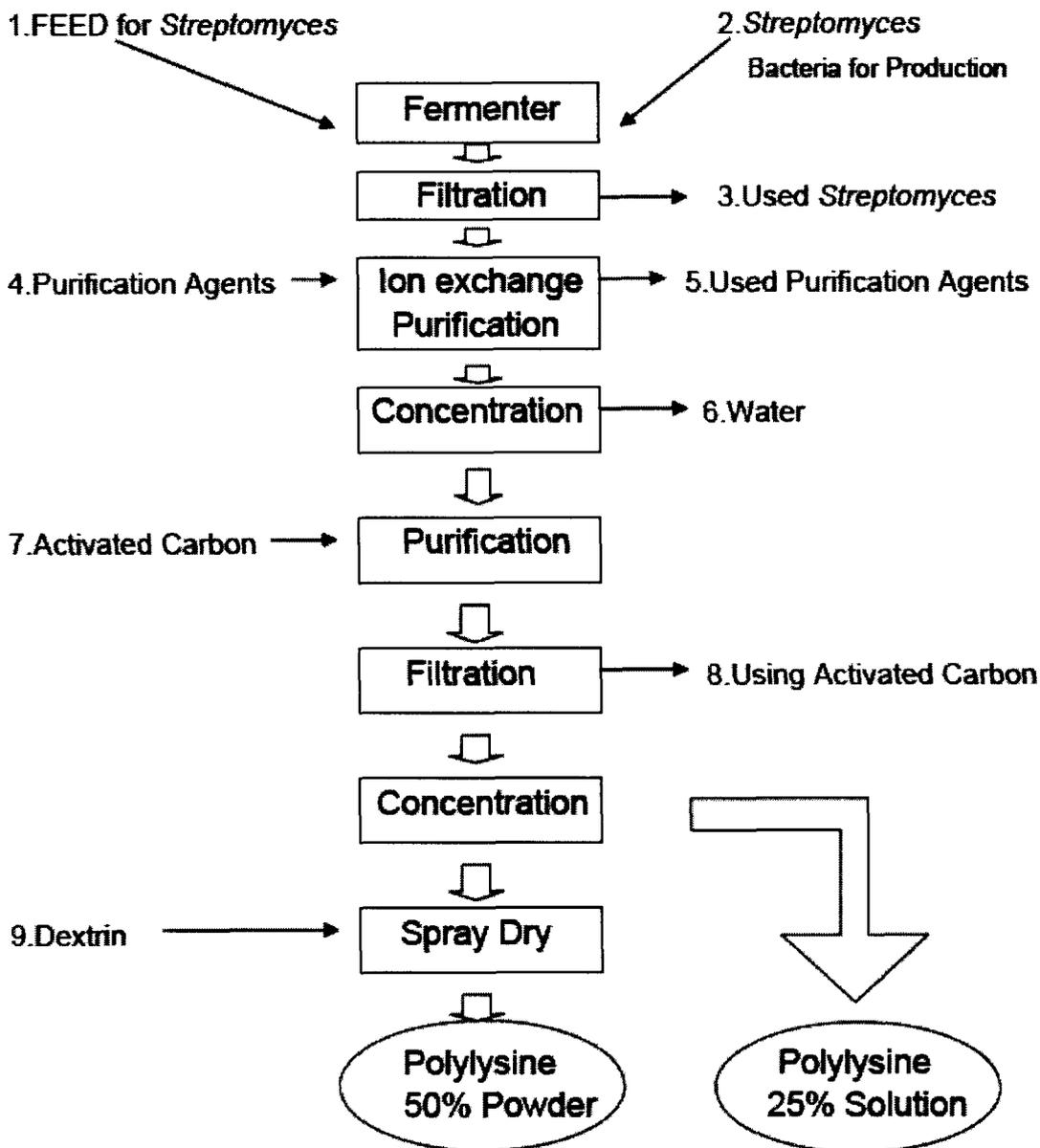
EPSILON-POLYLSINE GRAS NOTICE

1006, Rohm and Hass, eluent: water). Activated charcoal (trade name: Shirasaegi FA-SOW, Takeda Pharmaceutical Co.), is then used to clarify the product and remove any remaining organic impurities. Concentration of the eluent is accomplished using evaporative techniques, and the resulting ϵ -polylysine solids are then powdered or atomized to provide the desired fine powder product form.

Table II.B-1 Raw Materials and Processing Aids Used in the Manufacture of ϵ -Polylysine

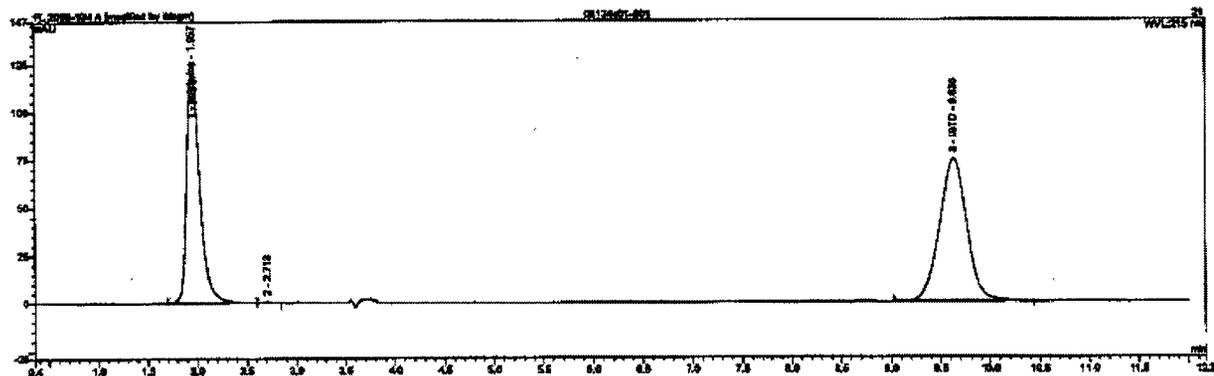
Material	Use	Regulatory Status
Raw Materials		
Glucose	Carbon substrate for fermentation	Permitted food ingredient (FCC, 2008)
Maltodextrin	Filler – Direct food ingredient	Permitted food ingredient (FCC, 2008)
Water	Solvent	N/A
Processing-Aids		
Activated Carbon (granular)	Purification-aid	No federal regulations specific to the intended use were identified Similar uses of activated carbon are considered GRAS for purification and clarification of wine as per 27 CFR §24.246 (U.S. ATTTB, 2009)
<u>Cation Column</u> <ul style="list-style-type: none"> Weakly acidic cation exchange resin 	Purification-aid	Used in accordance with 21 CFR §173.25 (U.S. FDA, 2009c)
<u>Anion Column</u> <ul style="list-style-type: none"> Strong basic anion exchange resin Polystyrene divinylbenzene copolymer Quarternary ammonium functional group ⁻OH counter ion 	Purification-aid	Used in accordance with 21 CFR §173.25 (U.S. FDA, 2009c)
<u>Reverse Phase Chromatographic Resin</u> Strongly acidic cation exchange resin <ul style="list-style-type: none"> Cross-linked polystyrene divinylbenzene copolymer Regenerated with NaOH 	Purification-aid	Used in accordance with 21 CFR §173.25 (U.S. FDA, 2009c)
<i>Streptomyces albulus</i> subsp. lysinopolymerus	Fermentation organism for synthesis of ϵ -polylysine	GRAS (GRN No. 135 – U.S. FDA, 2004) See Section IV.I for data supporting safe use of the organism

Figure II.B-1 Outline of Manufacturing Process for ε-Polylysine



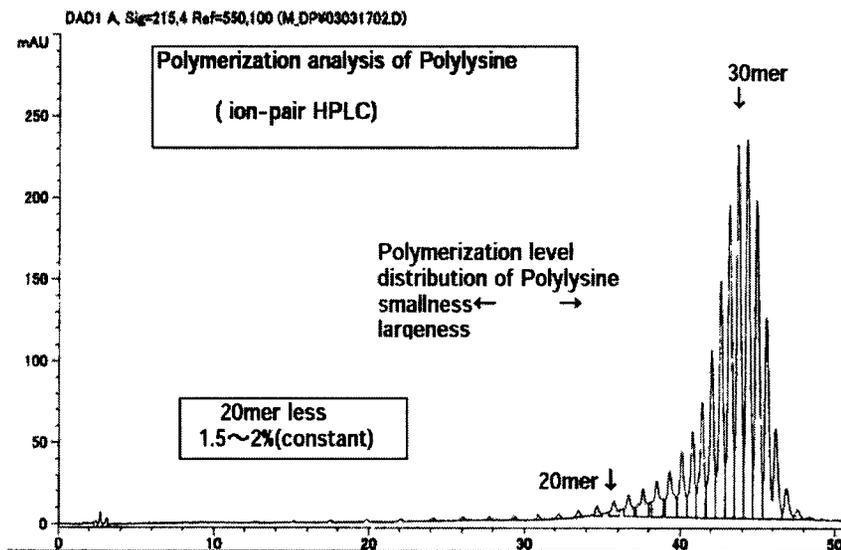
As shown in the high-performance liquid chromatography (HPLC) chromatogram in Figure II.B-1 below, the manufacturing process yields a highly pure polylysine product. The first peak corresponds to polylysine while the second peak corresponds to crotonic acid used as an internal standard.

Figure II.B-2 HPLC Chromatogram of Polylysine (Lot Number 2071001)



Extensive batch analysis of the final food grade product has been conducted to determine the molecular weight distribution of ϵ -polylysine, and numerous lots of the finished product have been analyzed *via* the ion pair HPLC method. This method generates a higher resolution of the molecular weight distribution of ϵ -polylysine than can be obtained using the traditional HPLC analysis shown in Figure II.B-2 above. As depicted below, a typical production batch of ϵ -polylysine produces a tight molecular weight distribution, with the majority of ϵ -polylysine polymers consisting of between 25 to 35 lysine residues (Figure II.B-3). Epsilon-Polylysine products with a polymerization of less than 20 residues are consistently below 2% of the total product.

Figure II.B-3 Ion-Paired HPLC Chromatogram of ϵ -Polylysine



C. Product Specifications

The product specifications for the ϵ -polylysine powder and aqueous formulations are shown below in Table II.C-1. The powdered formulation intended for market consists of 50:50 mixture of ϵ -polylysine and maltodextrin powder that will be sold under the trade name PuraQ Xtend FX50P. The second ϵ -polylysine formulation is a 25% w/v solution of ϵ -polylysine, and has been trade named PuraQ Xtend FX25. Various additional product formulations may be developed to meet particular customer needs. Batch analyses for the powder and aqueous ϵ -polylysine products are presented in Tables II.C-2 and II.C-3 below confirming that the ingredients contain the intended concentration of ϵ -polylysine, and are free of contaminating heavy metals, and microorganisms.

Table II.C-1 Specification for Purac Formulations: PuraQ Xtend FX50 P And PuraQ Xtend FX25

Parameter	Specification		Analytical Methods
	PuraQ Xtend FX50P 50:50 Mixture of ϵ -Polylysine and Maltodextrin Powder	PuraQ Xtend FX25 ϵ -Polylysine 25% Solution in Water	
Purity	50 to 54%	25 to 27%	HPLC*
Heavy Metals	≤ 10 ppm	≤ 10 ppm	ICP-OES
Lead	≤ 1.0 ppm	≤ 1.0 ppm	ICP-OES
Total Aerobic count (cfu/g)	≤ 500	≤ 500	HGMF
Yeasts and Moulds (cfu/g)	≤ 500	≤ 500	HGMF

*Internal validated method; ICP = Inductively Coupled Plasma; HGMF = Hydrophobic Grid Membrane Filtration Method

Table II.C-2 Batch Analyses: PuraQ Xtend FX50 P - 50:50 Mixture of ϵ -Polylysine and Maltodextrin Powder

Parameter	Specification	Lot Number		
		1081002	1080401	1080502
Purity	50 to 54%	51	51	52
Heavy Metals	≤ 10 ppm	<10	<10	<10
Lead	≤ 1 ppm	<1	<1	<1
Total Aerobic count (cfu/g)	≤ 500	<500	<500	<500
Yeasts and Moulds (cfu/g)	≤ 500	<500	<500	<500

Table II.C-3 Batch Analyses: PuraQ Xtend FX25 - ε-Polylysine 25% Solution In Water

Parameter	Specification	Lot Number		
		2081001	2081101	2081201
Purity	25 to 27%	26	25	26
Heavy Metals	≤10 ppm	<10	<10	<10
Lead	≤1 ppm	<1	<1	<1
Total Aerobic count (cfu/g)	≤500	<500	≤500	<500
Yeasts and Moulds (cfu/g)	≤500	<500	≤500	<500

D. Stability

As reported by Shih *et al.* (2006) ε-polylysine is a highly stable compound. The authors reported that ε-polylysine is stable in solution at elevated temperature and at low pH. The antimicrobial activity of ε-polylysine is dependent upon its molecular size. The relationship between polymer chain length and the antimicrobial effect of ε-polylysine against *Escherichia coli*-K12 has been reported by Shima *et al.* (1984). The authors demonstrated that effective inhibition of microbial growth requires a polymerization of at least 9-lysine residues, as the octamer displayed negligible antimicrobial activity against *E. coli*-K12. This observation indicates that the chemical and corresponding functional stability of ε-polylysine can effectively be determined through experiments characterizing the antimicrobial effects of ε-polylysine against common food pathogens under experimental conditions representative of various food uses. Studies evaluating the stability of ε-polylysine in solution under conditions of increasing temperature and over various pH ranges have been conducted by Hiraki (2000). As shown in Table II.D-1, subjecting ε-polylysine to elevated temperatures in solution does not affect the antimicrobial capacity of ε-polylysine against *E. coli*. Similarly ε-polylysine is an effective antimicrobial against *Bacillus subtilis*, *Bacillus cereus*, *E. coli*, and *Staphylococcus aureus* over a pH range of 5 to 8 (Table II.D-2). Poor stability is reported under alkaline conditions (≥pH 9); however, highly alkaline foods are uncommon, and were not identified under the proposed food uses. The available stability information was determined to support the suitability of the proposed food uses described herein (see Section IV.D).

Table II.D-1 Effect of Temperature on the Minimum Inhibitory Concentration (MIC) of ε-Polylysine Against Growth of *Escherichia coli* (adapted from Hiraki, 2000)

Treatment Condition	MIC (µg/mL)
None	50
80°C, 60 min	50
100°C, 30 min	50
120°C, 20 min	50

Table II.D-2 Effect of pH on the Minimum Inhibitory Concentration (MIC) of ϵ -Polylysine Against Growth of Various Pathogens (adapted from Hiraki, 2000)

Bacteria	MIC ($\mu\text{g/mL}$)				
	5 pH	6 pH	7 pH	8 pH	9 pH
<i>Bacillus subtilis</i>	3	3	3	3	12.5
<i>Bacillus cereus</i>	2.5	5	30	12.5	>200
<i>Escherichia coli</i>	25	25	50	50	>200
<i>Staphylococcus aureus</i>	12.5	25	12.5	<6.3	>200

III. SELF-LIMITING LEVELS OF USE

Self-limiting levels of use of ϵ -polylysine are imposed by the bitter flavor of the compound, which renders a food unpalatable when applied at high concentration. The threshold concentration at which ϵ -polylysine renders food unpalatable has not been determined.

IV. BASIS FOR GRAS DETERMINATION

ϵ -Polylysine was first introduced in Japan, where it is approved for use as a preservative in boiled rice and various traditional dishes (sukiyaki, noodle soup stocks, noodles and cooked vegetables). In 2003, ϵ -polylysine was introduced to the U.S. marketplace by the Chisso Corporation under the self-affirmation GRAS process for use as a preservative in rice products at a level of 0.005%. This GRAS use of ϵ -polylysine was reviewed by a Panel of Experts⁴ who concluded that the proposed use of ϵ -polylysine was GRAS. A no objection letter for this use was issued by the FDA in 2004, as described in GRAS Notification 135.

Purac wishes to extend the GRAS uses of ϵ -polylysine to include numerous additional food types⁵ at increased use levels of up to 0.025%. A use of up to 0.06% for meat and poultry products also is proposed. The purpose of this dossier is to present information supporting the safety and suitability of expanding the food uses and uses levels of ϵ -polylysine, and to provide information indicating that such uses are GRAS. The ϵ -polylysine ingredient that is the subject of this Notification is manufactured by Chisso Corp.; therefore, the majority of information supporting the GRAS evaluation of the proposed uses of ϵ -polylysine has been excerpted directly from the original GRAS notification as provided to the FDA (U.S. FDA, 2004). To

⁴ Drs. Douglas Archer; W. Gary Flamm; Donald A. Hughes.

⁵ Alcoholic beverages; baked goods and baking mixes; beverages and beverage bases; cheeses; coffee and tea; condiments and relishes; dairy product analogs; egg products; fats and oils; fish products; fresh eggs; fresh meats; fresh poultry; fruit and water ices; gelatins, puddings, and fillings; grain products and pastas; gravies and sauces; meat products, milk and milk products; nuts and nut products; plant protein products; poultry products; processed fruits and fruit juices; processed vegetables and vegetable juices; soups and soup mixes; sweet sauces, toppings and syrups.

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ensure that information reviewed is current, and includes all relevant information, an updated literature search was conducted to identify relevant information available in the public domain published since the original GRAS determination.

The data and information summarized in this dossier demonstrate that ϵ -polylysine, produced by Chisso Corp. via a fermentation process involving *Streptomyces albulus* subsp. *lysinopolymerus*, using cGMP and meeting appropriate food-grade specifications, is GRAS, based on scientific procedures, under the conditions of intended use in foods as described herein.

The information used to support the GRAS use of Purac's ϵ -polylysine preservative under the proposed uses described in Table A-1, is based on a series of published studies evaluating the toxicity and metabolic fate of ϵ -polylysine in rodents, and on the history of safe use of ϵ -polylysine as a preservative in various food types in the United States and Japan. Specifically, these safety studies summarized below consist of investigations of the acute, sub-chronic, chronic toxicity, and *in vitro* genotoxicity of ϵ -polylysine. This information was originally published in peer-reviewed Japanese journals and have been summarized by Hiraki *et al.* (2003). Published reproductive and teratology studies also are reviewed. The metabolic fate of ϵ -polylysine has been reported in the literature. Using radiolabeled ϵ -polylysine, Hiraki *et al.* (2003), have demonstrated that the compound displays limited bioavailability in rodents, which was attributed to the compounds poor absorption from the digestive tract. The published references supporting the safety of ϵ -polylysine were conducted with ϵ -polylysine produced by the Chisso Corporation, and since Purac sources its ϵ -polylysine from Chisso, the studies described below are considered directly relevant to the safety of ϵ -polylysine under the proposed uses.

Moreover, these data were reviewed by a Panel of Experts, qualified by scientific training and experience to evaluate the safety of ϵ -polylysine as a food ingredient, who concluded that the aforementioned proposed uses of ϵ -polylysine are safe and suitable and would be GRAS based on scientific procedures (see Appendix B for copy of the Expert Panel Statement). A summary of the data reviewed by Purac is presented herein.

A. Background Exposure to ϵ -Polylysine in the Diet

ϵ -Polylysine does not occur naturally in the diet, and there are no federal regulations pertaining to the use of ϵ -polylysine in food. The use of ϵ -polylysine as a preservative/anti-microbial agent for addition to cooked rice and sushi rice at a use level of up to 0.005% has been self-affirmed as GRAS by Chisso Corporation. Following notification to the FDA, the Agency stated that it "has no questions at this time regarding Chisso's conclusion that polylysine is GRAS for use as an antimicrobial agent in cooked rice or sushi rice at levels up to 50 mg/kg" (GRN No. 135 – U.S. FDA, 2004). As described in by the Notifier (Chisso Corp.), the estimated maximum

EPSILON-POLYLYSINE GRAS NOTICE

consumption of ϵ -polylysine under the conditions of use in rice and sushi rice, would result in an exposure of 15 mg per person per day in a nominal 60 kg individual.

B. Intended Uses

The intended use of ϵ -polylysine is to serve as an antimicrobial agent or preservative as defined in 21 CFR §170.3 (0)(2), which refers to substances used to preserve food by preventing growth of microorganisms and subsequent spoilage (U.S. FDA, 2009c).

Several investigators have evaluated the antimicrobial effects of ϵ -polylysine against various pathogenic microorganisms, and in various food matrices, and these data support the suitability of the use of ϵ -polylysine as a preservative under the proposed conditions of use. As shown by Yoshida and Nagasawa (2003), ϵ -polylysine has potent antimicrobial activity against a broad range of microorganisms (Table IV.B-1), and minimum inhibitory concentrations (MICs) of between 3 to 250 μg ϵ -polylysine/mL were reported by the authors. Furthermore, the antimicrobial effect of polylysine requires slightly acidic conditions, and at pH values of 8.0 or greater the antimicrobial effect is diminished significantly requiring much larger concentrations to elicit comparable effects to those observed at lower pH.

Table IV.B-1 Minimum Inhibitory Concentrations of ϵ -Polylysine Against Various Fungi and Bacteria (Yoshida and Nagasawa, 2003)

Organism	mg/mL	
Fungi	<i>Aspergillus niger</i> IFO4416	250
	<i>Trichophyton mentagrophytes</i> IFO7522	60
	<i>Candida acutus</i> IFO1912	6
	<i>Phaffia rhodozyma</i> IFO10129	12
	<i>Pichia anomala</i> IFO0146	10
	<i>P. membranaefaciens</i> IFO0577	3
	<i>Rhodotorula lactase</i> IFO1423	25
	<i>Sporobolomyces roseus</i> IFO1037	3
	<i>Saccharomyces cerevisiae</i>	50
	<i>Zygosaccharomyces rouxii</i> IFO1130	150
Gram Positive Bacteria	<i>Geobacillus stearothermophilus</i> IFO12550	5
	<i>Bacillus coagulans</i> IFO12583	10
	<i>B. subtilis</i> IAM1069	3
	<i>Clostridium acetobutylicum</i> IFO13948	32
	<i>Leuconostoc mesenteroides</i> IFO3832	50
	<i>Lactobacillus brevis</i> IFO3960	10
	<i>L. plantarum</i> IFO12519	5
	<i>Micrococcus luteus</i> IFO12708	16
	<i>Staphylococcus aureus</i> IFO13276	12
	<i>Streptococcus lactis</i> IFO12546	100

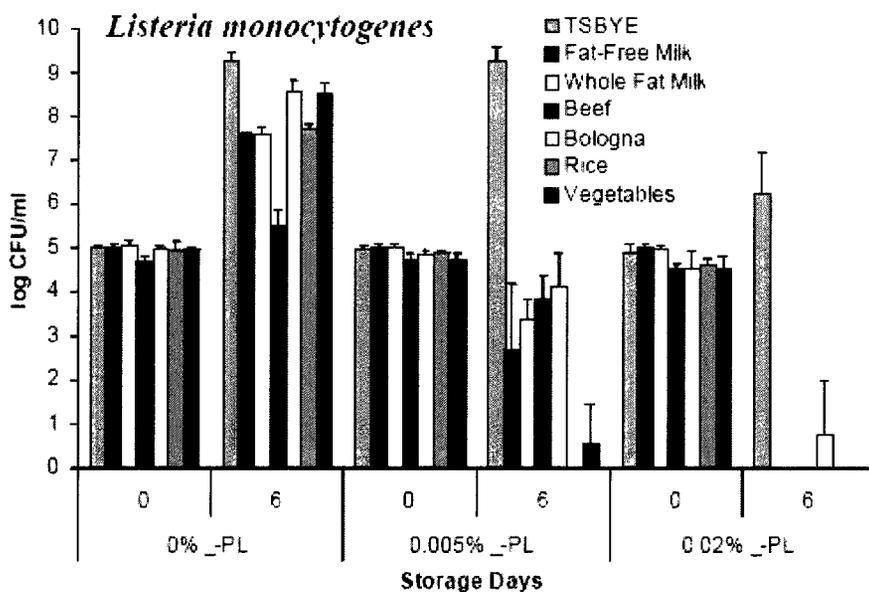
Table IV.B-1 Minimum Inhibitory Concentrations of ε-Polylysine Against Various Fungi and Bacteria (Yoshida and Nagasawa, 2003)

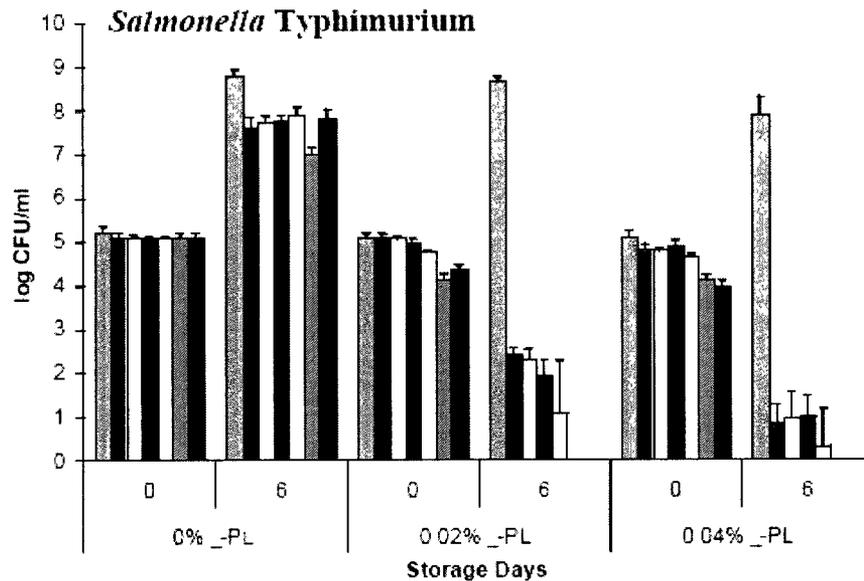
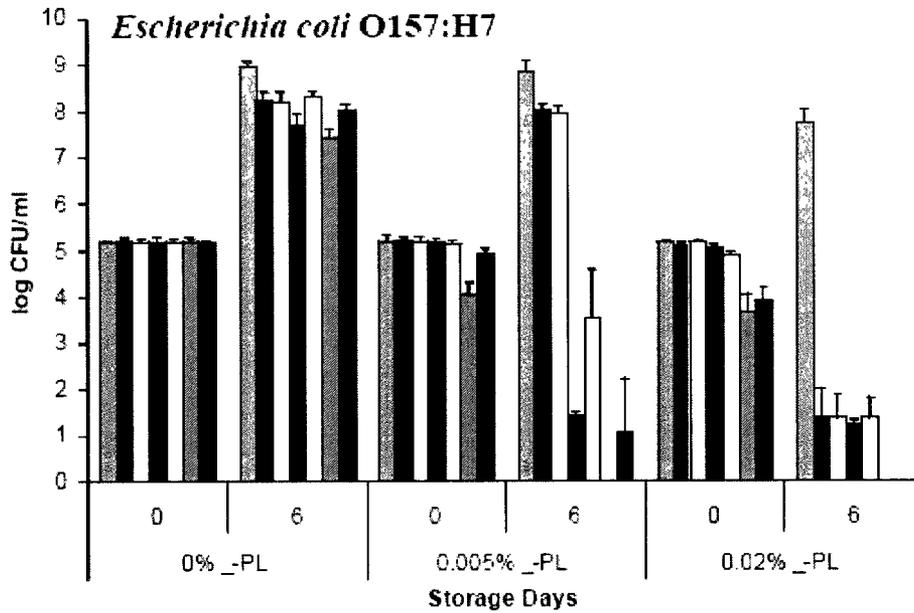
Organism	mg/mL	
Gram Negative Bacteria	<i>Raoultella planticola</i> IFO3317	8
	<i>Campylobacter jejuni</i> 100	100
	<i>Escherichia coli</i> IFO13500	50
	<i>Pseudomonas aeruginosa</i> IFO3923	3
	<i>Salmonella typhimurium</i>	16

The antimicrobial efficacy of ε-polylysine in various food matrices was evaluated by Geornaras *et al.* (2007). As illustrated in Figure IV.B-1 below, ε-polylysine at concentrations of up to 0.02%, exerted significant bactericidal effects in fat-free milk, whole fat milk, beef extract, bologna extract, rice extract and vegetable extract samples over a 6-day period.

The mechanism by which ε-polylysine induces its potent antibacterial effects is not completely understood; however, most antimicrobial peptides operate *via* bactericidal and bacteristatic effects that are mediated by physical ionic interactions with the microbial cell wall/membranes inducing pore formation or disintegrating the cell membrane. These effects are distinct from the mechanism(s) by which clinical antibiotics operate and are therefore suitable for food uses, where the development and spread of antibiotic resistance is a concern.

Figure IV.B-1 Preservative Effects of ε-Polylysine in Various Food Types. Mean (log CFU/mL) populations of *L. monocytogenes*, *E. coli* O157:H7, and *S. typhimurium* in inoculated (5 log CFU/mL) tryptic soy broth supplemented with 0.6% yeast extract (TSBYE), or food extract (10% w/w in distilled water), in the presence and absence of ε-polylysine (ε-PL), stored at 53.6°F for 6 days (from Geornaras *et al.*, 2007).





C. Estimated Intake of ϵ -Polylysine from the Proposed Food Uses

The estimated total intake of polylysine from all proposed food-uses in the U.S. by population group is summarized in Table IV.C-1. Table IV.C-2 presents these data on a per kilogram body weight basis.

One-hundred percent of the total U.S. population was identified as consumers of polylysine from the proposed food-uses (8,008 actual users identified). As a result of the high number of users identified with all population groups, the intake assessments for the all-person and all-user categories were similar and therefore only the all-user results are discussed in detail.

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Consumption of these types of foods by the total U.S. population resulted in estimated mean all-user intake of polylysine of 283 mg/person/day, equivalent to 4.5 mg/kg/day on a body weight basis (Tables IV.C-1 and IV.C-2). The 90th percentile all-user intake of polylysine from all proposed food-uses by the total population was observed to be 520 mg/person/day, or 8.4 mg/kg body weight/day.

Table IV.C-1 Summary of the Estimated Daily Intake of Polylysine from All Proposed Food Uses in the U.S. by Population Group (2003-2004 NHANES Data)

Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption		All-Users Consumption	
				Mean (mg)	90 th Percentile (mg)	Mean (mg)	90 th Percentile (mg)
Infants	0 to 2	72.3	672	71	168	89	182
Children	3 to 11	100.0	1,287	192	342	192	342
Female Teenagers	12 to 19	100.0	992	251	423	251	423
Male Teenagers	12 to 19	100.0	999	336	569	336	569
Female Adults	20 and Up	100.0	2,129	262	473	262	473
Male Adults	20 and Up	100.0	1,929	354	608	354	608
Total Population	All Ages	96.9	8,008	280	517	283	520

USDA, 2009

Table IV.C-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Polylysine from All Proposed Food Uses in the U.S. by Population Group (2003-2004 NHANES Data)

Population Group	Age Group (Years)	% Users	Actual # of Total Users	All-Person Consumption		All-Users Consumption	
				Mean (mg/kg)	90 th Percentile (mg/kg)	Mean (mg/kg)	90 th Percentile (mg/kg)
Infants	0 to 2	72.3	672	5.7	13.4	7.2	14.8
Children	3 to 11	100.0	1,287	7.1	13.0	7.1	13.0
Female Teenagers	12 to 19	100.0	992	4.3	7.5	4.3	7.5
Male Teenagers	12 to 19	100.0	999	5.2	8.5	5.2	8.5
Female Adults	20 and Up	100.0	2,129	3.7	6.8	3.7	6.8
Male Adults	20 and Up	100.0	1,929	4.1	7.5	4.1	7.5
Total Population	All Ages	96.9	8,008	4.5	8.4	4.5	8.4

USDA, 2009

On an individual population basis, the greatest mean all-user intake of polylysine on an absolute basis was observed to occur in male adults, at 354 mg/person/day. Infants displayed the lowest mean all-user intake of polylysine on an absolute basis, with a value of 89 mg/person/day. On a body weight basis, the mean all-user intake of polylysine was highest in infants, with a value of

7.2 mg/kg body weight/day. The lowest mean all-user intake on a per kilogram body weight basis was observed in female adults, with a value of 3.7 mg/kg body weight/day (Table IV.C-2).

When heavy consumers (90th percentile) were assessed, all-user intake of polylysine from all proposed food-uses also was determined to be greatest in male adults at 608 mg/person/day. The lowest 90th percentile all-user intake was again observed to occur in infants, with a value of 182 mg/person/day on an absolute basis (Table IV.C-1). On a body weight basis, the highest all-user 90th percentile intake of polylysine was estimated for infants, with a value of 14.8 mg/kg body weight/day (Table IV.C-2). The lowest all-user 90th percentile intake of polylysine on a body weight basis was observed in female adults, with a value of 6.8 mg/kg body weight/day.

D. Absorption, Distribution, Metabolism, and Elimination

The pharmacokinetics and metabolism of ϵ -polylysine has been investigated by Hiraki *et al.* (2003). Eighteen (18) male Crj:CD Sprague-Dawley rats (7 weeks of age; 250 to 289 g) were obtained from Charles River Japan, and following a 1-week acclimatization period, were assigned to 1 of 4 experimental groups: 3 rats to Group 1 for determination of radioactivity in blood and plasma; 9 rats to Group 2 for determination of parent compound and low molecular weight compounds in plasma; 3 rats in Group 3 for determination of radioactivity in urine, feces, expired air and residual carcass; and three rats to Group 4 for whole body autoradiography. Rats were fasted from the previous day prior to dosing and resumed feeding 4 hours post-dosing. Aqueous dosing solutions were formulated to contain 20 mg/mL ¹⁴C-labeled ϵ -polylysine, and rats were dosed orally by gavage with volumes delivering 100 mg ϵ -polylysine/kg body weight. The ϵ -polylysine used in the study was provided by the Chisso Corporation. Animals used for blood and plasma collections were held as groups of 3 in standard caging. For collection of urine, feces, and expired air, animals were housed in glass metabolism cages with wire mesh bottoms and cages and were washed with distilled water to recover urine radioactivity. Carbon dioxide in expired air was collected in dual traps containing 20% aqueous monoethanolamine. Serial blood samples were obtained from the tail vein of rodents in Group 1, and blood samples from animals in Group 2 were obtained during exsanguination *via* the abdominal aorta. The design of the absorption, distribution, metabolism, excretion (ADME) study is summarized in the following Table IV.D-1 with collection times and biological samples taken.

Table IV.D-1 Group Designations, Dose and Collections for ADME Study

Group	Number Animals (males)	Test Article Study Parameters	Target Dose (mg/kg bw)	Total Radioactivity (MBq/kg)	Collections Matrix and Times (hours post-dosing)
1	3	¹⁴ C-ε-polylysine radioactivity in blood and plasma	100	3 7	Blood 0-1, 1-2, 2-4, 4-6, 6-8, 8-24, 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168
2	9	¹⁴ C-ε-polylysine parent and metabolites in plasma	100	7 4	Blood 0-8, 8-24, and 24-12
3	3	¹⁴ C-ε-polylysine excretion in urine, feces and expired air	100	3 7	Urine and CO ₂ in expired air 0-4, 4-8, 8-24, 24-48, 48-12, 12-96, 96-120, 120-144, 144-Feces 0-24, 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168 Carcass. 168
4	3	¹⁴ C-ε-polylysine whole body autoradiography	100	7 4	Whole body autoradiography 8, 24 and 168

Blood and plasma samples in Group 1 were dissolved in Soluene-350 tissue solubilizer (Packard) and decolorized with benzyl peroxide. Plasma samples in Group 2 were processed by adding 1% ammonia water/methanol to plasma followed by sonication and centrifugation. The supernatant was then collected and the procedure was repeated twice more. Urine samples from 3 animals were combined and diluted with distilled water for scintillation counting or evaporated to dryness and reconstituted in solvent for HPLC analysis. Combined fecal samples from all animals in a group were homogenized prior to centrifugation to separate the fecal-bound from free radioactivity prior to radiation counting. Samples were then added to scintillation solutions or analyzed by HPLC as appropriate. For whole body autoradiography, animals were frozen in dry ice-acetone and 35 µm frozen sections were prepared. Sections were covered with a protective membrane and stored on imaging plates (TYPE-BAS, Fuji Film). The radioactive images were analyzed by a Bio-image device (FUJIX BAS2000, Fuji Film) to prepare color radiolumigrams. Data on absorption and elimination of radioactivity from the blood and plasma pool following oral administration of 100 mg ¹⁴C-labeled ε-polylysine are presented in Table IV.E-1. Since all carbons were radiolabeled, these data do not necessarily show the presence of ε-polylysine, but reflect the radioactivity concentrations equal to microgram equivalents (µg eq.) of labeled ε-polylysine carbons. The t_{max} in blood was 7.3 hours, and in plasma, 8.0 hours, suggesting a slow or delayed absorption of radioactivity. The maximum concentrations estimated for blood and plasma were 3.16 and 5.13 µg eq. ε-polylysine/mL, which occurred 6 to 8 hours post-dosing. Half-lives for elimination from blood and plasma over the 72- to 168-hour period were 20 and 3.9 days, respectively, which presumably reflects the metabolic utilization of L-lysine *via* its incorporation into various proteins. These prolonged half-lives are likely due to a significant proportion of the early blood and plasma radioactivity being derived from absorbed L-lysine amino acid cleaved from

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ϵ -polylysine by proteases in the upper intestinal tract, which may be produced by the resident microflora. Upon absorption, L-lysine is readily incorporated into longer-lived peptides and proteins that are released and persist in circulation. Less than half of the peak radioactivity (5.13 $\mu\text{g eq. } \epsilon$ -polylysine/mL) in plasma at 8 hours was found in plasma at 48 hours (2.23 $\mu\text{g eq. } \epsilon$ -polylysine/mL), but the blood radioactivity at termination (168 hours) had not declined to half the 8-hour peak. Area under-the-curve (AUC) values for blood and plasma over the 0- to 168-hour period were not significantly different.

Table IV.D-2 Concentration of Radioactivity in Blood and Plasma: ϵ -Polylysine Equivalents

Time	Radioactivity concentration ($\mu\text{g eq of } \epsilon$ -polylysine/mL)	
	Blood Mean (SD)	Plasma Mean (SD)
30 min	ND	0.240 (0.014)
1 hr	0.337 (0.059)	0.499 (0.061)
2	1.014 (0.079)	1.456 (0.144)
4	2.438 (0.247)	3.848 (0.535)
6	3.039 (0.728)	4.994 (1.339)
8	2.963 (1.089)	5.134 (1.240)
24	2.273 (0.548)	3.277 (0.815)
48	2.169 (0.565)	2.225 (0.498)
72	2.079 (0.496)	1.843 (0.464)
96	1.844 (0.392)	1.464 (0.362)
120	1.817 (0.426)	1.244 (0.257)
144	1.808 (0.388)	1.010 (0.317)
168	1.716 (0.286)	0.908 (0.163)
Detection limit	0.178	0.198
t_{max} (hr)	7.3 (1.2)	8.0 (0.0)
C_{max} ($\mu\text{g eq./mL}$)	3.158 (0.889)	5.134 (1.240)
$t_{1/2}$ (72-168 hr) (day)	20 (6)	3.9 (0.4)
AUC (0-168 hr)	339 (79)	330 (78)
($\mu\text{g eq. hr/mL}$) ($0-\infty$)	1470 (270)	543 (87)

Date are expressed as the mean values (Standard Deviation) of 3 animals; ND = not detected

The cumulative excretion of radioactivity in urine, feces, and expired air, as well as residual activity in the carcass, are presented in Table IV.D-3. The overall mean recovery from all routes and biological samples was approximately 100% of the administered radioactivity. The majority of the radioactivity was recovered in the feces within the first 24 hours (71.5%), by 48 hours, 92.9% of the dose was found cumulatively in the feces and practically all subsequent elimination in the feces was complete by 72 hours. In expired air, approximately 50% of the total cumulative amount excreted in the urine (3.4% of the dose) was eliminated over the 168-hour collection period within the first 8 hours and approximately 90% of the total within 48 hours

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(3.0% of the dose). Little radioactivity was found in the urine during the first 8 hours. By 48 hours 1.2% of the administered dose, corresponding to 57% of the cumulative collection was excreted. Through 168 hours, the cumulative radioactivity in the urine totaled 2.1% of the administered dose. The residual carcass had only 0.9% of the dose, and the amount of radioactivity recovered during cage washing was minimal. As these data show, the entire dose of radioactivity was eliminated by excretion within 168 hours and over 97% was accounted for in urine, feces or expired air by 48 hours, mainly in the feces (92.9%). The remaining sum of the cumulative excretion with routes associated with absorption, urine, expired air and residual carcass activity, is 6.4% of total recovered radioactivity. The results of this study demonstrate that ϵ -polylysine is poorly bioavailable due to first pass exclusion by the gastrointestinal tract; approximately 94% of the 100 mg/kg body weight dose of ϵ -polylysine passes unabsorbed through the gastrointestinal tract and is eliminated in the feces following oral administration of the compound to rodents.

Table IV.D-3 Cumulative Excretion of Radioactivity in Urine, Feces and Expired Air

Time (hr)	Excretion of Radioactivity (% of dose) (SD)			
	Urine	Feces	Expired Air	Total
0-4	0.0 (0.0)	--	0.3 (0.1)	--
8	0.1 (0.1)	--	1.7 (0.7)	--
24	0.8 (0.7)	71.5 (11.6)	2.6 (0.8)	75.0 (0.8)
48	1.2 (0.7)	92.9 (0.8)	3.0 (0.8)	97.1 (0.8)
72	1.5 (1.0)	94.1 (1.5)	3.1 (0.9)	98.7 (0.2)
96	1.7 (1.1)	94.2 (1.5)	3.2 (0.8)	99.1 (0.4)
120	1.9 (1.2)	94.3 (1.5)	3.3 (0.8)	99.5 (0.5)
144	2.0 (1.3)	94.4 (1.5)	3.4 (0.9)	99.7 (0.7)
168	2.1 (1.5)	94.4 (1.5)	3.4 (0.9)	100.0 (0.9)
Carcass (168 hr)				0.9 (0.1)
Cage washing (168 hr)				0.0 ± 0.1

Date are expressed as the mean values (SD) of 3 animals
 -- = not determined

An additional metabolism study was then conducted by the authors to characterize the metabolic by-products in plasma and feces (Hiraki *et al.*, 2003). A single male rodent (Crj:CD Sprague-Dawley rat) was administered ¹⁴C radiolabeled polylysine as described above, at a dose of 100 mg/kg body weight, blood was collected at 30 minutes and 4 and 8 hours after dosing, feces were collected from 0 to 24 hours. Samples were extracted with methanol and subject to HPLC for evaluation of the various radioactive fractions based on molecular weight. Control sample recoveries from plasma were reduced over time, reported as 83%, 25%, and 20% at 30 minutes, and 4 and 8 hours respectively (Hiraki *et al.*, 2003). Polylysine, oligopeptides (4 mer and 6 mer) were synthesized and utilized as comparative control compounds during the HPLC analyses. Measurements of radioactivity above the detection limit were observed only for polylysine tetramer (0.006 µg/mL; 0.8% of total radioactivity in plasma)

at 30 minutes post-dosing. At 4 hours, neither polylysine nor any of its oligomer degradation products were noted. At 8 hours, plasma levels of the 6 mer were 0.033 µg/mL (0.6% of total radioactivity) and ε-polylysine was 0.012 µg/mL (0.2% of plasma radioactivity); no tetramers were detectable. Overall, less than 1% of the absorbed radiolabel was in the form of polylysine or its oligomeric fragments. In the feces, 0.7% (1% of total plasma counts) of the administered dose was recovered as ε-polylysine, 0.3% (0.5% of total plasma counts) of the tetramer, and 19% (2.8% of total plasma counts) as a 6 oligopeptide over the 24-hour collection period. Since only 8% of the radioactivity could be recovered in the supernatant of centrifuged feces, most of the polylysine or its metabolites were considered to be bound to the fecal solids (Hiraki *et al.*, 2003). Next, the authors investigated the metabolic profile of ε-polylysine by administering ¹⁴C-L-lysine at a dose of 100 mg/kg to male rats (Crj:CD Sprague-Dawley rat) by gavage and collecting blood samples at 30 minutes and 4 hours. Plasma samples were extracted with methanol for HPLC analysis; recoveries of radioactivity from samples were 77.6% and 9.4% for the 30-minute and 4-hour samples respectively. At 30 minutes, 67.2% of the radioactivity in plasma corresponded to L-lysine (48.7 pg/mL) and at 4 hours, 7.5% of the radioactivity appeared to be L-lysine. These data suggest that absorbed L-lysine is readily incorporated into protein or further metabolized. Earlier reports have shown that lysine is degraded to α-aminoadipate through the saccharopine intermediate in rat liver mitochondria (Higashino *et al.*, 1967; Grove *et al.*, 1970). Studies of L-lysine catabolism in guinea pigs and rats have also shown the presence of α-aminoadipate, which is deaminated to another reported metabolite, α-ketoadipate in these animals (Rodwell, 1969). Therefore, a reasonable interpretation is that the metabolism of ε-polylysine proceeds primarily by cleavage of L-lysine amino acid from the homopolymer with 4 mer and 6 mer fragment formation. The L-lysine is further degraded to the α-aminoadipate and α-ketoadipate metabolites that are each found in plasma at levels comparable to that observed when ¹⁴C L-lysine is administered. It is possible that both gut microflora and the liver are capable of degrading L-lysine and contributing to the α-aminoadipate and α-ketoadipate metabolites found in plasma.

E. Toxicity Studies

(i) Acute Toxicity Studies

Crj:CD Sprague-Dawley rats (4 weeks of age; 10/sex/dose group) were administered 10% ε-polylysine in water at dose levels of 1.25, 2.5, and 5.0 g ε-polylysine/kg body weight by gavage (Hiraki *et al.*, 2003). Male rodents weighed between 118 to 131 g, and females between 104 to 116 g at the beginning of the experiment (Dosing Day 0). The test article used in the study was provided by the Chisso Corporation, and was formulated as a 50:50 ratio of ε-polylysine and maltodextrin powder. Animals were not fasted prior to treatment. Body weights were taken on Days 0, 3, 7, and 14 post-dosing. The animals were observed for 14 days after treatment for general appearance, behavioral changes, and signs of toxicity. After the 2-week observation period, the animals were euthanized and examined for gross

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pathological changes. With the exception of a slight depression in spontaneous activity in rats administered the highest dose, which returned to normal at 3 to 6 hours, no clinical signs of toxicity were observed. No differences in weight gain were found related to treatment during the 14-day observation period; however, a dose-dependent trend for reduced weight gain was observed in both sexes on Day 1 post-dosing (~10% reduction relative to control groups). Animals regained their normal growth rates between Days 3 and 14. No deaths were reported. At necropsy, there were no abnormal findings in any major organ (Hiraki *et al.*, 2003). Therefore, the acute LD₅₀ is greater than 5.0 g ε-polylysine/kg body weight by gavage or practically non-toxic.

(ii) Subchronic Toxicity Studies

The subchronic oral toxicity of ε-polylysine was evaluated by Ishii *et al.* (1993). Sixty male and female Crj:CD Sprague-Dawley rats 4 weeks of age were obtained from Charles River Japan. After a 10-day acclimatization period, 40 of the 60 rodents were randomized into 1 of 4 groups each containing 10 animals per sex. Rodents were housed in pairs in stainless steel bracket cages under controlled environmental conditions (temperature 19 to 22°C, humidity of 45 to 65% and 12 air changes per hour). Animals were administered diets containing 2,000, 10,000, and 50,000 ppm ε-polylysine in the diet for 90 days, corresponding to a mean daily intake of 179 or 193, 895 or 995, and 4,295 (males) or 4,779 (females) mg/kg body weight at the low-, mid-, and high-dose groups respectively. The test article used in the study was provided by the Chisso Corporation, and was formulated as a 50:50 ratio of ε-polylysine and maltodextrin powder. Body weight and food consumption was measured weekly and general animal condition was monitored daily. At termination, standard urinalysis⁶ and hematological analysis⁷ including differential leukocyte count and prothrombin times were obtained. Blood clinical chemistry parameters⁸ also were obtained, as well as pre-dosing and post-dosing ophthalmological examinations. Following gross observations of the organs, organ weights were taken on the liver, kidneys, brain, pituitary, thyroid, submaxillary gland, thymus, heart, spleen, adrenals, testes, seminal vesicle, prostate, uterus and ovary. In addition to these organs, numerous other tissues⁹ were collected for subsequent histopathological examination.

At 50,000 ppm, male and female rats showed significant decreases in food consumption beginning at Weeks 1 to 5, and significant depression of weight gain during the first week of treatment. The reduction in weight gain was attributed to the poor palatability of the bitter

⁶ occult blood, ketone, glucose, protein, pH, urobilinogen, bilirubin, nitrite, sodium, potassium, urinary sediment, urine volume, specific gravity and color.

⁷ erythrocytes, leukocytes, thrombocytes, and hematocrit level.

⁸ Serum, GOT, GPT, ALP, LDH, glucose, total cholesterol, uric acid, triglycerides, phospholipids, total protein, albumin, total bilirubin, urea nitrogen, creatine, inorganic phosphorus, calcium, A/G ration, sodium, potassium, and chloride levels.

⁹ Tongue, parathyroid, trachea, bronchus, pancreas, urinary bladder, sternum and femur, spine, ischiadic nerve, muscle (femoral muscle), skin, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, mesenteric lymph node, aortic arch, vagina, harder gland, epididymis, and mammary gland.

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tasting polylysine resulting in reduced food intake. No significant decreases in weight gain, food, or water consumption, were seen among animals consuming the test article at concentrations of 10,000 ppm and below. Rats of both sexes had decreased potassium excretion at the high dose, with females also having reduced volume and increased specific gravity. Urinalysis was unaltered by treatment in the lower dose groups. Male rats at 50,000 ppm had a significant decrease in leukocyte count and increase in segmented leukocyte ratio. In the lower-dose groups, sporadic changes in hematocrit, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin and lymphocyte or segmented lymphocyte ratios were noted. The hematological changes in all treatment groups were not considered to be of toxicological significance since they were not dose dependent, were slight in magnitude and within the historical ranges for controls. Further, no histopathology was seen in any part of the hematopoietic system including spleen, bone marrow, lymph ducts, and thymus gland. Males and females showed significant decreases in blood glucose, total protein, albumin, triglyceride and phospholipid levels and male rats had lowered cholesterol. These changes were thought to be secondary to reduced food consumption. Other groups had sporadic, non dose-dependent changes in biochemistry parameters that were considered to be within historical ranges and not toxicologically significant. Due to the reduced weight gain seen in the high-dose group males and females, several organ weights were found to be reduced on an absolute basis relative to controls. The relative organ weights were reduced only for the liver in mid- and high-dose males and females and for the thyroid in all female treatment groups. With the exception of a diffuse slight atrophy of hepatocytes in animals treated at 50,000 ppm, no histopathological abnormalities related to treatment were observed in any dose group. Based on the findings in this subchronic feeding study, a no-observed-effect level of 10,000 ppm in the diet (895 mg/kg body weight in males and 995 mg/kg body weight in females) was determined by the authors. This NOEL determination was concluded to be appropriate.

(iii) Chronic

Following the subchronic study, the chronic oral toxicity and carcinogenicity of ϵ -polylysine was investigated by Fukotome *et al.* (1995). Three hundred and fifty (350) male and female Crj:Sprague-Dawley rats (4 weeks of age) were obtained from Charles River Japan. After a 14-day acclimatization period, 320 of the 350 rats were randomized to 1 of 4 groups containing 80 male and 80 female rodents per group. Housing conditions and ϵ -polylysine test article formulation were as described in the subchronic toxicity study (Ishii *et al.*, 1993). Animals were administered diets containing 2,000, 6,500, and 20,000 ppm ϵ -polylysine for a duration of 102 to 104 weeks¹⁰. This corresponded to a mean daily intake for males or females respectively of approximately 100 or 122, 332 or 417, and 1,060 or 1,317 mg/kg body weight at the low-, mid-,

¹⁰ The original study design was based on a 130-week treatment interval; however, due to animal mortality the experiment was terminated when the cumulative fatality ratio reached 75%, resulting in termination of the experiment at weeks 102 (males) and 104 (females).

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and high-dose groups. Body weight and food consumption was measured weekly through Week 14 and monthly thereafter. General animal condition was monitored daily and palpable masses were noted and monitored on a weekly basis after Week 26. For 10 rats/sex/group at every 13 weeks during the study, and on all survivors at termination, standard urinalyses, hematological analyses, clinical chemistry were obtained as described by Ishii *et al.* (1993). Gross observations of the organs, and organ weights were obtained for the liver, kidneys, brain, spleen, adrenals, and testes on Weeks 26, 52, and 78 for 10 rats/sex/group as well as all survivors at termination. In addition, numerous other tissues as described by Ishii *et al.* (1993) were collected for subsequent histopathological examination at these time points.

Suppression of weight gain in the high dose groups, attributed by the authors to poor palatability of the test article, was observed at Week 1 to 5 and Weeks 7 through 64 of treatment in male rats, and similar observations were reported for the females at Weeks 1, 11, 13 to 28, and 36 to 48. At termination, the body weights were not significantly different between treated and control groups. No treatment related effects on body weight or feed consumption were observed in males or females consuming the test article at the low and mid-dose concentrations at any time. No increased incidence of abnormal clinical signs or reduced survival was observed. Although sporadic alterations of some parameters were found, no treatment related changes in hematology, blood biochemistry, or urinalysis were reported. Gross observations and organ weights were unremarkable. All non-neoplastic and neoplastic lesions observed in the treatment animals did not show dose-dependent increases, and were considered to be spontaneous in origin as comparable incidences of neoplastic and non-neoplastic lesions were observed in the control groups. Based on the experimental findings of suppressed weight gain and decreases in food consumption efficiency in male and female rats consuming the high dose diets (20,000 ppm), the authors derived a no-observed-effect level (NOEL) of 6,500 ppm (male: 332 mg/kg body weight/day; female: 417 mg/kg body weight/day) when chronically administered in the diet. However, consistent with the findings in the sub-chronic study, the reduced weight gain was attributed to poor palatability of the diet. Based on the observations that the reduced weight gain was not dose-responsive, that biochemical, hematological and histopathological findings were unremarkable, and that body weights reverted to normal by the end of the study, a no-observed-adverse-effect-level (NOAEL) of 20,000 ppm (1,059 or 1,317 mg/kg in males or females respectively) in the diet, the highest dose administered can be determined. This conclusion is consistent with the conclusions of Hiraki *et al.* (2003) and that of the previous Expert Panel during the original GRAS notification (GRN No. 135 – U.S. FDA, 2004).

(iv) Reproduction and Teratogenicity

The reproductive toxicity and teratogenicity of ϵ -polylysine was evaluated by Neda *et al.* (1999). Crj:CD Sprague-Dawley rats¹¹ were administered ϵ -polylysine in the diet at concentrations of 0, 3,000, 10,000, or 30,000 ppm. The effect of ϵ -polylysine on reproductive functions including estrous cycles, copulation, fertilization, parturition and lactation, growth of offspring, and development of embryos or fetuses for 2 generations was investigated.

In the F₀ males, a slight reduction in weight gain relative to controls was observed. Since the reduction in weight gain is consistent with palatability issues reported in previous studies at comparable doses by Ishii *et al.* (1993) and Fukotome *et al.* (1995), was not dose responsive, and did not occur in females, the finding was not considered adverse. No treatment related abnormalities in estrous cycles, mating and fertility for males and females, pregnancy, parturition, lactation, weaning and necropsy was reported in the 30,000 ppm groups. Polylysine had no effects on weight gain or food and water consumption during pregnancy or lactation. No abnormalities were observed in the lower dose groups.

In the F₁ generation, body weight gains in the 30,000 ppm groups were suppressed in both sexes during the 98-day treatment period; corresponding information on food intake were not available from the Japanese article. Although the authors reported that vaginal opening was delayed in this generation, the effect does not appear to be a biologically relevant finding as significant effects on indices of fertility are not reported in the tabulated data. In this generation, the consumption of polylysine at a dietary concentration of 30,000 ppm was not associated with treatment related adverse effects on the following parameters: clinical observation at birth, external morphology, viability, weaning, behavioral function, neurotoxicological tests, immunotoxicological tests, estrous cycles, mating and fertility for males and females, pregnancy, lactation, numbers of corpora lutea and implants, pre-implantation loss, parturition, weaning index, gross necropsy findings, organ or body weights and food consumption. No abnormalities were observed in the lower dose groups.

Similar to findings reported by the authors for previous generations, body weights were reported to be decreased in male and female animals randomized to the high-dose polylysine diet (30,000 ppm) by Postnatal Day 21. Confirmation of this finding was not possible as empirical data for this endpoint is not presented in English, nor was corresponding information on the food consumption of these animals available. There were no treatment related abnormalities in the clinical observations at birth, external morphology, viability, growth and differentiation, behavioral functions, necropsy and organ weights. No adverse effects were reported in the lower-dose groups. Teratology evaluations revealed no treatment related abnormalities at cesarean section, including observations of external, skeletal, and visceral examinations.

¹¹ Male rodents were between 208.8 to 263.5 g and females were between 145.7 to 176.6 g at the beginning of study. Housing and environmental conditions were as described in the subchronic and chronic studies.

The authors concluded that ϵ -polylysine at dietary concentrations of up to 30,000 ppm in the diet, the highest dietary concentration administered, did not cause any adverse effects on reproductive, neurological, and immunological function, or embryonic and fetal development and growth.

F. Mutagenicity and Genotoxicity Studies

Epsilon-polylysine was tested for mutagenicity in the Ames *Salmonella* tester strains TA 1535, TA 1537, TA98, and TA100 and *E. coli* WP2 uvrA with and without S-9 metabolic activation. Due to inhibition of growth in range finding assays, the highest concentrations tested were 1.2 $\mu\text{g}/\text{plate}$ in *S. typhimurium* TA 1537 and 4.9 $\mu\text{g}/\text{plate}$ for the other 4 strains without metabolic activation. With metabolic activation, the maximum dose for the *S. typhimurium* strains was 1,250 and 313 $\mu\text{g}/\text{plate}$ for *E. coli* WP2 uvrA. When tested at these doses ϵ -polylysine did not induce any significant or dose-responsive increase in revertants indicative of mutagenic activity (Hiraki *et al.*, 2003).

G. Human Studies

Studies containing relevant safety endpoints related to the consumption of ϵ -polylysine by humans were not identified in the public domain. Unpublished studies with safety related endpoints conducted in humans also were not available.

H. Allergenicity

No reports were found in the literature of any allergenic reactions or other adverse effects associated with the use of ϵ -polylysine in food, even with common use in Japan. The structure of ϵ -polylysine is not typical of proteinaceous compounds in that it consists of a polymer with only single amino acid constituent versus the normal presence of multiple different amino acids in protein molecules. The likelihood of allergenicity to ϵ -polylysine is expected to be low.

I. Safety of *Streptomyces albulus* subsp. lysinopolymerus

(i) Taxonomy and History of Use

Epsilon-polylysine is manufactured using a fermentation process catalyzed by *Streptomyces albulus* subsp. lysinopolymerus. *Streptomyces albulus* is a member of the *Streptomyces* genus that contains a number of bacterial species that have a history of use in the industrial production of several enzymes, which have found application in the food industry in the U.S., and world-wide. For example, under 21 CFR §184.1372 several uses of insoluble glucose isomerase enzyme preparations derived from recognized species of precisely classified, nonpathogenic, and nontoxicogenic microorganisms of *Streptomyces rubiginosus*, *Streptomyces olivaceus*, and *Streptomyces olivochromogenes* grown in a pure culture fermentation that produces no

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antibiotic have been GRAS affirmed by the FDA. Vitamin B₁₂ obtained from *Streptomyces griseus* also has been GRAS affirmed for use as a nutrient in a number of foods (21 CFR §184.1945). Finally, the food additive natamycin derived from *Streptomyces natalensis* and *Streptomyces chattanoogensis* are permitted for use as a mycotoxin in various foods (21 CFR §172.155) (U.S. FDA, 2009c).

A summary of the current taxonomic assignment (based on the 8 kingdom classification scheme) of *S. albulus* subsp. *lysinopolymerus* is presented in Table IV.I-1.

Taxonomy	Taxonomic Assignment
Kingdom	Bacteria
Subkingdom	Bacteria
Phylum	Actinobacteria
Class	Actinobacteria (class)
Order	Actinobacteridae
Family	Streptomycetaceae
Genus	<i>Streptomyces</i>
Species	<i>Streptomyces albulus</i>
Subspecies	<i>lysinopolymerus</i>
Strain	1101A-1

A search of the publicly available literature did not reveal articles, or other information suggesting that members of the species *S. albulus* exhibit pathogenic or toxicogenic phenotypes.

(ii) Antibacterial By-Products

Epsilon-polylysine molecules are cationic, surface active agents, a function of their positively charged amino groups. The molecule has hydrophobic methylene groups on the interior of the molecule and exterior hydrophilic carboxyl and amino groups. Cationic surface-active compounds antagonize the proliferation of microorganisms and ϵ -polylysine has well established antimicrobial properties. Actinomycetes such as *Streptomyces albulus* are known to produce multiple antibacterial substances besides ϵ -polylysine, and a number of *Streptomyces* species are sources of pharmaceutical antibiotics. Antibacterial products with mechanisms of action similar to those used in clinical practice ("antibiotics") are not permitted for use in food. Therefore, experiments were conducted to determine the presence and quantity of any novel antibiotic substances in the final polylysine product (Chisso, 2001). To identify novel antibiotic compounds, the fermentation media was fractionated on silica gel after removal of polylysine and tested for antibacterial activity on paper discs against *B. cereus*, *E. coli*, and *S. aureus*. Two fractions, designated fraction A and B were found to have zones of inhibition on

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agar plates demonstrating antibacterial activity. A minimal inhibitory concentration of 0.25% w/w against *B. cereus* and *S. aureus* was determined for both fractions. The fractions were then analyzed using HPLC to obtain peak retention times for the unknown antibacterial constituents. The detection limits for the chromatographic peaks were then determined following admixture of each fraction with various concentrations of polylysine. The limits of detection were determined to be 1.1 µg/mL for fraction A (quantitative limit of 3.2 µg/mL) when added to a 2,000 µg/mL solution of ε-polylysine, and 0.045 µg/mL for fraction B (quantitative limit of 0.14 µg/mL) when added to a 400 µg/mL solution of ε-polylysine. Neither of the antibacterial compounds present in fraction A or B was detected during subsequent HPLC analyses of the purified ε-polylysine product. Based on the limit of detection for the antibacterial constituents of fractions A and B, these compounds are not expected to be present in the ε-polylysine product at concentrations that would produce antimicrobial activity.

J. Summary and Basis for GRAS

Epsilon-polylysine has been used as a food preservative for a number of years in Japan, where it is permitted for use in a variety of food applications including boiled rice, noodle soup stocks, noodles and cooked vegetables. There are no reports of adverse effects in the literature associated with the food use of ε-polylysine. The compound has recently been introduced to the U.S. marketplace, and as described in GRAS Notification GRN No. 135 (submitted by the Chisso Corporation), ε-polylysine is self-affirmed as GRAS for use as a preservative in rice and sushi rice at a use level of 0.005% (U.S. FDA, 2004). Purac proposes to expand the current GRAS use of ε-polylysine to include a number of additional food categories and use levels as described in Table A1.

The ε-polylysine used in Purac's ingredient is a food grade product obtained from the Chisso Corp., and is manufactured in accordance with cGMP, using a non-toxicogenic non-pathogenic strain of *Streptomyces albulus* subsp. *lysinopolymerus* as described in the original GRAS self-affirmation (Chisso Corporation; GRAS Notification No. 135 – U.S. FDA, 2004). Briefly, the procedure involves an aerobic fermentation process using non-milk based fermentation media, with glucose used as the carbon source for ε-polylysine synthesis. Upon completion of the fermentation stage, the organism is removed from the fermentation media *via* filter sterilization (0.1 µm filter), and a purified solution of ε-polylysine is then produced using a series of sequential anion, and cation exchange steps followed by a chromatographic purification. Two ingredients are intended for market, a powdered formulation consisting of a 50:50 mixture of ε-polylysine and food grade maltodextrin, and an aqueous formulation of ε-polylysine diluted in water to a concentration of 25% w/v. The respective trade-names for these products are PuraQ Xtend FX50P and PuraQ Xtend FX25. Consistent with the quality requirements expected of a food grade material, both ingredients are characterized to a high purity of ~100% using HPLC analysis, and contain appropriate limitations on lead and microbial contaminants (Table A3-1, Attachment 3). Batch analyses from 3 non-consecutive lots of the aqueous and powder

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formulations indicate that these products are produced in a consistent manner in compliance with product specifications. ϵ -Polylysine is a highly stable compound, and based on information obtained from the literature ϵ -polylysine retains its antimicrobial activity in solution under conditions of elevated temperatures and over a pH range of 4 to 8. Poor stability is reported under alkaline conditions (\geq pH 9); however, highly alkaline foods are uncommon, and were not identified under the proposed food uses. The available stability information was determined to support the suitability of the proposed food uses described herein.

Under the expanded food uses 100% of the total U.S. population was identified as consumers of polylysine from the proposed food-uses. Consumption of these types of foods by the total U.S. population resulted in estimated mean all-person and all-user intakes of polylysine of 280 mg/person/day (4.5 mg/kg body weight/day) and 283 mg/person/day (4.5 mg/kg body weight/day), respectively. The 90th percentile all-person and all-user intakes of polylysine from all proposed food-uses by the total population were observed to be 517 mg/person/day (8.4 mg/kg body weight/day) and 520 mg/person/day (8.5 mg/kg body weight/day), respectively. When heavy consumers (90th percentile) were assessed, all-user intake of polylysine from all proposed food-uses also was determined to be greatest in male adults at 608 mg/person/day. The lowest 90th percentile all-user intake was observed to occur in infants, with a value of 182 mg/person/day on an absolute basis. The highest all-user 90th percentile intake of polylysine was observed in individuals aged 0 to 2 years, with a value of 14.8 mg/kg body weight/day. Background dietary exposure to ϵ -polylysine from the current GRAS uses are limited to cooked rice and sushi rice at a use level of up to 50 ppm. The estimated maximum consumption of ϵ -polylysine under the conditions of use in rice and sushi rice was estimated to result in a maximum exposure of 15 mg/person/day in a nominal 60 kg individual. Therefore, the estimated intake from the proposed uses is not appreciably affected by the current background uses.

Published information pertaining to the safety of ϵ -polylysine under the proposed uses described herein were based on the same information used to determine the GRAS status of the ingredient as described in the original GRAS self-affirmation (Chisso Corp; GRAS Notification No. 135 – U.S. FDA, 2004). An updated literature search of the public domain did not identify any information contradicting the previous GRAS conclusion. Several additional studies not available during the original GRAS determination were identified in the literature, and include data relevant to the suitability of ϵ -polylysine under the expanded uses and use levels of ϵ -polylysine. These published studies describe the antimicrobial effects of ϵ -polylysine against a broad range of important food borne pathogens (*e.g.*, *E. coli*, *Clostridium* sp., *Salmonella* sp., *Bacillus* sp.). Studies supporting the suitability of ϵ -polylysine under different food matrices, although not comprehensive, also provide additional support for the suitability of the ingredient under the proposed food uses. These studies indicate that ϵ -polylysine displays significant antimicrobial activity under conditions relevant to the proposed use levels.

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Several pharmacokinetic and metabolism studies were conducted in Crj:CD SD rats administered radiolabeled ϵ -polylysine at acute gavage doses of 100 mg/kg body weight (Hiraki *et al.*, 2003). The results of these studies indicate that ϵ -polylysine is poorly bioavailable due to its limited absorption and first pass-exclusion by the gut mucosa. Ninety-four percent of the administered dose (100 mg/kg body weight) was eliminated in the feces unabsorbed within 48 hours. The remaining 6% was excreted by routes that involved absorption; 3% was released as $^{14}\text{CO}_2$ in expired air, 2% was found in urine and 1% remained in the carcass. Based on whole body autoradiography findings no evidence of organ specific radiolabel accumulation was observed, and by 168 hours post dosing, only trace levels of radioactivity were found in the tissues and organs. The absorption of radioactivity from the stomach and upper gastrointestinal tract was slow; maximum concentrations of ϵ -polylysine equivalents in the blood and plasma occurring at 8 hours were in the range of 3 to 5 $\mu\text{g}/\text{mL}$. Although the plasma values declined by 50% from the 8-hour peak values within 40 hours, longer half-lives of 3.9 days were calculated for plasma and 20 days for blood. The authors attributed this finding to the absorption of small quantities of radiolabeled L-lysine cleaved from the homopolymer by proteases in the upper intestinal tract. Proteases also may be produced by bacteria in the gastrointestinal tract as ϵ -polylysine-degrading hydrolytic enzymes of bacterial origin (*Sphingobacterium multivorum*) have been identified in the literature (Kito *et al.*, 2002). Upon absorption, L-lysine is readily incorporated into longer-lived peptides and proteins, which are released and persist in circulation. Detailed analysis of HPLC profiles from the metabolic studies by the authors support this interpretation; the predominant metabolite was L-lysine, and only 0.2% of the administered parent compound was present in the plasma.

The subchronic and chronic toxicity of dietary ϵ -polylysine has been evaluated in rodents (Ishii *et al.*, 1993; Fukotome *et al.*, 1995)¹². During the subchronic study, male and female Crj:CD SD rats (5 to 6 weeks of age) were administered ϵ -polylysine at concentrations of 2,000, 10,000, and 50,000 ppm in the diet for 90 days (Ishii *et al.*, 1993). Reduction in body weight and corresponding reductions in organ weights were observed in the high-dose males and females. These effects were associated with poor diet palatability. Mild histopathological changes in the liver also were reported in the high-dose groups. Due to a number of statistically significant changes in various biochemical, hematological, and histopathological findings, which were limited to male and females consuming the high dose diets (50,000 ppm; 4,295 mg/kg/day), a NOEL of 10,000 ppm in the diet (895 mg/kg body weight in males and 995 mg/kg body weight in females) can be determined.

The chronic toxicity of ϵ -polylysine was evaluated using 320 Crj:CD SD rats (6 weeks of age) administered ϵ -polylysine in the diet at concentrations of 2,000, 6,500, and 20,000 ppm for up to 102 and 104 weeks respectively for male and females (Fukotome *et al.*, 1995). No differences

¹² The articles were published in peer-reviewed Japanese journals, and contained English tables, figures, and abstracts. Additionally, English translations of these articles were provided to the Expert Panel.

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in animal survival between treatment groups in either sex were observed. No increases in the incidence of non-neoplastic or neoplastic lesions attributable to the test article were reported. Neither the liver or any other organ or tissue showed a treatment-related effect at any dose level following histopathological examinations. No consistent or dose-related alteration of clinical biochemistry, urinalysis, or hematology was associated with treatment in the chronic feeding study. The only statistically significant finding reported by the authors was a transient reduction in weight gain during the mid-phase of the study in males, which recovered to control levels by 84 weeks. A reduction in weight gain, notably in male animals, is a consistent finding that has been reported in all of the toxicology studies conducted with polylysine to date. Polylysine is well established to be bitter at high concentrations. During these studies, the observed reductions in weight gain are reported in animals consuming between 20,000 to 50,000 ppm in the diet, and in all cases were limited to the highest dietary concentration with no evidence of dose responsive effects across large margins of food intakes. In addition, the reductions in weight gain also were typically associated with corresponding reductions in food intake. These observations are consistent with the criteria highlighted by Flamm *et al.* (2003) as representing critical factors whereby reduced weight gain is attributed to palatability issues. This conclusion also is consistent with World Health Organization's (WHO) guidance (WHO, 1987) concerning the interpretation of lower body weight gain in the absence of other toxicity due to consumption of a test material with known nutritive and palatability effects. Thus, a NOAEL of 20,000 ppm in the diet or 1,060 mg/kg, which was the lower value ingested by the male rats (1,317 mg/kg in high-dose females), can be determined. The previous Expert Panel also considered a NOAEL of 20,000 ppm in the diet to be an appropriate determination for this study.

A two-generation reproduction and teratogenicity study conducted in Crj:CD SD rats administered ϵ -polylysine in the diet (0, 3,000, 10,000, or 30,000 ppm) was reported by Neda *et al.* (1999)¹³. No treatment related effects on reproductive, neurological, and immunological function, or embryonic and fetal development and growth were noted at any dose.

In vitro studies evaluating the mutagenicity of ϵ -polylysine were conducted using the bacterial reverse mutation assay in the presence and absence of metabolic activation using Ames tester strains *S. typhimurium* TA100, TA1535, TA98, and TA1537, as well as *E. coli* strain WP2 uvrA. Although the tests were limited to low doses due to the antimicrobial properties of the compound, no mutagenic effects were observed. These observations are consistent with the absence of neoplastic effects reported in the chronic toxicity evaluation.

The GRAS use of *S. albulus* subsp. *lysino*polymerus, also was considered. It was determined non pathogenic, non-toxicogenic strains of *S. albulus* *lysino*polymerus were GRAS for use in the synthesis of polylysine based on the following information: (1) ϵ -Polylysine is manufactured

¹³ Study was published in peer-reviewed Japanese journal, and contained English tables, figure, and abstract; English translation was not available.

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under cGMP employing suitable controls to prevent transfer of the fermentation organism to the final product (0.1 μm filter sterilization, followed by several ion-exchange purification steps). (2) Microbial analyses of the purified ϵ -polylysine solution prior to spray drying, confirms that the fermentation strain is absent in the final product. (3) No evidence was identified in the literature indicating that members of *S. albulus* subsp. lysinopolymerus are potentially pathogenic or toxicogenic. (4) Studies demonstrating the absence of *Streptomyces* antibiotics in the product. (5) Finally, the safety of *S. albulus* subsp. lysinopolymerus for use in the production of a food grade ingredient also is supported by the absence of toxicity, mutagenicity, or carcinogenicity observed in the product specific rodent toxicology, and *in vitro* mutation studies.

Overall, the results of rodent toxicity studies and *in vitro* mutagenicity studies indicate that ϵ -polylysine is of low-toxicity and does not display genotoxic potential. Assuming a worst-case maximum exposure to polylysine from the proposed food uses, the daily total population all user intake was estimated to be 8.4 mg/kg body weight per day. Highest exposures were estimated for individual's aged 0 to 2 and 3 to 11, where 90th percentile all user exposures were 14.8 and 13.0 mg/kg body weight/day respectively. The safety of the proposed uses of ϵ -polylysine in food is based on the significant margin of safety between the estimated exposures relative to the totality of evidence obtained from the available safety data. This conclusion was supported by the absence of toxicity reported in several rodent toxicity studies including the chronic administration of ϵ -polylysine to rats at a dose of up to 1,020 mg/kg body weight (the highest dose tested); that the 90th percentile intake estimates are considered gross over estimations of the actual exposures; that ϵ -polylysine is not absorbed and virtually no systemic exposure to the compound is expected when consumed in the diet under the proposed food uses. Thus, Purac concluded that provided ϵ -polylysine is manufactured in accordance with Good Manufacturing Practices (GMP), using suitable food grade ingredients, that the proposed expanded uses of ϵ -polylysine in food are GRAS based on scientific procedures.

K. Conclusion

Based on the data and information summarized above, it can be concluded that Purac's ϵ -polylysine product produced *via* a fermentation process using *S. albulus* subsp. lysinopolymerus, meeting appropriate food-grade specifications and manufactured in accordance with cGMP, is GRAS for the intended use as a preservative in traditional food products as described herein based on scientific procedures.

Therefore, the GRAS uses of ϵ -polylysine in food as described in this Notification are exempt from the requirement of premarket approval (Section 409 of the *Federal Food, Drug and Cosmetic Act*) (U.S. FDA, 2009b).

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EPSILON-POLYLSINE GRAS NOTICE

Table of CFR Sections Referenced (Title 21—Food and Drugs)		
Part	Section §	Section Title
101—Food labeling	101.12	Reference amounts customarily consumed per eating occasion
170—Food additives	170.3	Definitions
	170.30	Eligibility for classification as generally recognized as safe (GRAS)
172—Food additives permitted for direct addition to food for human consumption	172.155	Natamycin (pimaricin)
173— Secondary direct food additives permitted in food for human consumption	173.25	Ion-exchange resins
184—Direct food substances affirmed as generally recognized as safe	184.1372	Insoluble glucose isomerase enzyme preparations
	184.1945	Vitamin B&bdi1;&bdi2

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APPENDIX A

PROPOSED FOOD USES OF ϵ -POLYLYSINE IN THE U.S.

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Table A-1 Summary of the Individual Proposed Food-Uses and Use-Levels for Polylysine in the U.S.

Food Category	Proposed Food-Uses	Use-Levels (%)*
Alcoholic Beverages	Light Beer	0.01
	Wine	0.005
Baked Goods and Baking Mixes	Cake Batter and Cheesecakes	0.01
	Muffins and Popovers	0.01
	Pancakes and Waffles	0.025
	Pies (Not Fruit)	0.025
	Sweet Pastries	0.01 to 0.025
Beverages and Beverage Bases	Energy, Fitness, Sports, and Isotonic Drinks (RTD)	0.025
	Gelatin, Rice, and Sugar Cane-Based Beverages	0.025
	Soft Drinks	0.025
Cheeses	Cheese-Based Mixtures	0.025
	Cottage, Ricotta, and Cream Cheese	0.025
	Natural (Feta, Mozzarella, and Mexican)	0.025
	Processed Cheese and Cheese Spreads	0.025
Coffee and Tea	Coffee and Coffee Beverages	0.025
	Tea and Tea Beverages	0.025
Condiments and Relishes	Pickles, Olives, and Pickled Products	0.025
Dairy Product Analogs	Cream Substitutes	0.025
	Imitation Cheese	0.025
Egg Products	Egg-Based Dishes	0.025
Fats and Oils	Reduced-Fat or Fat-Free Margarine-like Spread	0.005
	Salad Dressings (Regular and Low-fat)	0.025
Fish Products	Fish and Shellfish-Based Foods	0.025
Fresh Eggs	Eggs	0.025
Fresh Fish	Fish and Shellfish	0.025
Fresh Meats	Beef (Steak, Stewing, and Ground)	0.06
	Pork (Chops and Steaks)	0.06
Fresh Poultry	Chicken Breast and Turkey	0.06
Fruit and Water Ices	Frozen Fruit Bars and Sorbet	0.025
Gelatins, Puddings, and Fillings	Gelatin	0.025
	Pie Fillings	0.025
	Puddings, Custards, and Mousses	0.025
Grain Products and Pastas	Grain-Based Dishes	0.025
	Pastas	0.025
Gravies and Sauces	Fat-Based Sauces	0.01
	Gravy	0.025
	Tomato-Based Sauces	0.025
Meat Products	Meat-Based Prepared Foods	0.02 to 0.03

Table A-1 Summary of the Individual Proposed Food-Uses and Use-Levels for Polylysine in the U.S.

Food Category	Proposed Food-Uses	Use-Levels (%)*
Milk and Milk Products	Chocolate-Flavored Drinks	0.01
	Cream	0.01
	Eggnog	0.01
	Sour Cream and Dips	0.025
	Yogurt	0.01
Nuts and Nut Products	Peanut Butters and Sauces	0.025
Plant Protein Products	Meat Substitutes	0.025
	Soy Products	0.025
Poultry Products	Poultry-Based Prepared Foods	0.025
Processed Fruits and Fruit Juices	Fruit-Flavored Drinks and Ades (RTD)	0.025
	Fruit Smoothies	0.025
	Nectars	0.025
	Prepared Fruit and Fruit Mixtures	0.025
Processed Vegetables and Vegetable Juices	Dry Beans and Bean-Based Prepared Foods	0.025
	Potato- and Sweet Potato-Based Foods (Excluding French Fries)	0.025
	Vegetable-Based Prepared Foods	0.01 to 0.025
	Tomato-Based Juices	0.025
Soups and Soup Mixes	Soups (Excluding Canned)	0.025
Sweet Sauces, Toppings, and Syrups	Sweet Syrups and Toppings	0.025

RTD = Ready-to-Drink.

* Use levels were based on 200 mg polylysine /RACC; RACC = Reference Amounts Customarily Consumed per Eating Occasion (21 CFR §101.12 – U.S. FDA, 2009b). When a range of values is reported for a proposed food-use, particular foods within that food-use may differ with respect to their RACC.

APPENDIX B

**EXPERT PANEL CONSENSUS STATEMENT CONCERNING
THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS
OF ϵ -POLYLYSINE FOR USE AS A PRESERVATIVE IN FOOD**

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EXPERT PANEL CONSENSUS STATEMENT CONCERNING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF E-POLYLYSINE FOR USE AS A PRESERVATIVE IN FOOD

December 09, 2009

INTRODUCTION

At the request of Purac Biochem b.v. (Purac), an Expert Panel (the "Panel") of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information, and determine whether use of ϵ -polylysine as a preservative in the proposed foods described in Table A1-1 (see Attachment 1) is safe and suitable and would be Generally Recognized as Safe (GRAS) based on scientific procedures. The Panel consisted of: Dr. Ian C. Munro, Ph.D. (Cantox Health Sciences International), Dr. Stanley M. Tarka, Ph.D. (The Tarka Group, Inc.), and Dr. John A. Thomas, Ph.D. (Indiana University School of Medicine). These Panel members have been determined to be qualified by relevant experience and scientific training to evaluate the safety of ϵ -polylysine under the proposed food uses. *Curricula vitae* for each Panel member are included in Attachment 2.

The Panel, independently and collectively, critically examined a comprehensive package of scientific information and data on ϵ -polylysine from the literature and other published sources. In addition, the Panel evaluated other information deemed appropriate or necessary. The information evaluated by the Panel included details pertaining to the method of manufacture and product specifications, supporting analytical data, intended use conditions of ϵ -polylysine as a preservative, estimated exposure, and a comprehensive assessment of the available scientific literature pertaining to the safety of ϵ -polylysine.

Following independent, critical evaluation of such data and information, the Panel convened on Tuesday October 6, 2009 and unanimously concluded that the intended use described herein for ϵ -polylysine, meeting appropriate food-grade specifications as described in the supporting dossier [**Documentation Supporting the Evaluation of ϵ -Polylysine as Generally Recognized as Safe (GRAS) for Use as a Preservative in Food**] and manufactured according to current Good Manufacturing Practice (cGMP), are safe and suitable and GRAS based on scientific procedures. A summary of the basis for the Panel's conclusion is provided below.

SUMMARY

Epsilon-polylysine has been used as a food preservative for a number of years in Japan, where it is permitted for use in a variety of food applications including boiled rice, noodle soup stocks, noodles and cooked vegetables. There are no reports of adverse effects in the literature associated with the food use of ϵ -polylysine. The compound has recently been introduced to the U.S. marketplace, and as described in GRAS Notification GRN 135 (submitted by the Chisso Corporation), ϵ -polylysine is self-affirmed as GRAS for use as a preservative in rice and sushi rice at a use level of 0.005%. Purac proposes to expand the current GRAS use of ϵ -polylysine to include a number of additional food categories and use levels as described in Table A1-1.

The ϵ -polylysine used in Purac's ingredient is a food grade product obtained from the Chisso Corp., and is manufactured in accordance with cGMP, using a non-toxicogenic non-pathogenic strain of *Streptomyces albulus* subsp. *lysinopolymerus* as described in the original GRAS self-affirmation (Chisso Corporation; GRAS Notification No. 135). Briefly, the procedure involves an aerobic fermentation process using non-milk based fermentation media, with glucose used as the carbon source for ϵ -polylysine synthesis. Upon completion of the fermentation stage, the organism is removed from the fermentation media *via* filter sterilization (0.1 μm filter), and a purified solution of ϵ -polylysine is then produced using a series of sequential anion, and cation exchange steps followed by a chromatographic purification (See Figure A3-1, Attachment 3). Two ingredients are intended for market, a powdered formulation consisting of a 50:50 mixture of ϵ -polylysine and food grade maltodextrin, and an aqueous formulation of ϵ -polylysine diluted in water to a concentration of 25% w/v. The respective trade-names for these products are PuraQ Xtend FX50P and PuraQ Xtend FX25. Consistent with the quality requirements expected of a food grade material, both ingredients are characterized to a high purity of ~100% using HPLC analysis, and contain appropriate limitations on lead and microbial contaminants (Table A3-1, Attachment 3). Batch analyses from 3 non-consecutive lots of the aqueous and powder formulations indicate that these products are produced in a consistent manner in compliance with product specifications. Epsilon-polylysine is a highly stable compound, and based on information obtained from the literature ϵ -polylysine retains its antimicrobial activity in solution under conditions of elevated temperatures and over a pH range of 4 to 8. Poor stability is reported under alkaline conditions (\geq pH 9); however, highly alkaline foods are uncommon, and were not identified under the proposed food uses. The available stability information was determined to support the suitability of the proposed food uses described herein.

Under the expanded food uses 100% of the total U.S. population was identified as consumers of polylysine from the proposed food-uses. Consumption of these types of foods by the total U.S. population resulted in estimated mean all-person and all-user intakes of polylysine of 280 mg/person/day (4.5 mg/kg body weight/day) and 283 mg/person/day (4.5 mg/kg body weight/day), respectively. The 90th percentile all-person and all-user intakes of polylysine from

all proposed food-uses by the total population were observed to be 517 mg/ person/day (8.4 mg/kg body weight/day) and 520 mg/person/day (8.4 mg/kg body weight/day), respectively. When heavy consumers (90th percentile) were assessed, all-user intake of polylysine from all proposed food-uses also was determined to be greatest in male adults at 608 mg/person/day (7.5 mg/kg body weight/day). The lowest 90th percentile all-user intake was observed to occur in infants, with a value of 168 mg/person/day on an absolute basis. The highest all-user 90th percentile exposure to polylysine was observed in individuals aged 0 to 2 years, with an estimate of 14.8 mg/kg body weight/day. Exposure to ϵ -polylysine from the current GRAS uses are limited to cooked rice and sushi rice at a use level of up to 50 ppm, and therefore were not considered to appreciably increase the above exposure estimations.

Published information pertaining to the safety of ϵ -polylysine under the proposed uses described herein were based on the same information used to determine the GRAS status of the ingredient as described in the original GRAS self-affirmation (Chisso Corp; GRAS Notification No. 135). An updated literature search of the public domain did not identify any information contradicting the previous GRAS conclusion. Several additional studies not available during the original GRAS determination were identified in the literature, and include data relevant to the suitability of ϵ -polylysine under the expanded uses and use levels of ϵ -polylysine. These published studies describe the antimicrobial effects of ϵ -polylysine against a broad range of important food borne pathogens (*e.g.*, *E. coli*, *Clostridium* sp., *Salmonella* sp., *Bacillus* sp.). Studies supporting the suitability of ϵ -polylysine under different food matrices, although not comprehensive, also provide additional support for the suitability of the ingredient under the proposed food uses. These studies indicate that ϵ -polylysine displays significant antimicrobial activity under conditions relevant to the proposed use levels.

Several pharmacokinetic and metabolism studies were conducted in Crj:CD SD rats administered radiolabeled ϵ -polylysine at acute gavage doses of 100 mg/kg body weight (Hiraki *et al.*, 2003). The results of these studies indicate that ϵ -polylysine is poorly bioavailable due to its limited absorption and first pass-exclusion by the gut mucosa. Ninety-four percent of the administered dose (100 mg/kg body weight) was eliminated in the feces unabsorbed within 48 hours. The remaining 6% was excreted by routes that involved absorption; 3% was released as ¹⁴CO₂ in expired air, 2% was found in urine and 1% remained in the carcass. Based on whole body autoradiography findings no evidence of organ specific radiolabel accumulation was observed, and by 168 hours post dosing, only trace levels of radioactivity were found in the tissues and organs. The absorption of radioactivity from the stomach and upper gastrointestinal tract was slow; maximum concentrations of ϵ -polylysine equivalents in the blood and plasma occurring at 8 hours were in the range of 3 to 5 μ g/mL. Although the plasma values declined by 50% from the 8-hour peak values within 40 hours, longer half-lives of 3.9 days were calculated for plasma and 20 days for blood. The authors attributed this finding to the absorption of small quantities of radiolabeled L-lysine cleaved from the homopolymer by proteases in the upper intestinal tract. Proteases also may be produced by bacteria in the gastrointestinal tract as

ϵ -polylysine-degrading hydrolytic enzymes of bacterial origin (*Sphingobacterium multivorum*) have been identified in the literature (Kito *et al.*, 2002). Upon absorption, L-lysine is readily incorporated into longer-lived peptides and proteins, which are released and persist in circulation. Detailed analysis of HPLC profiles from the metabolic studies by the authors support this interpretation; the predominant metabolite was L-lysine, and only 0.2% of the administered parent compound was present in the plasma.

The subchronic and chronic toxicity of dietary ϵ -polylysine has been evaluated in rodents (Ishii *et al.*, 1993; Fukotome *et al.*, 1995)¹. These studies have been reviewed by Hiraki *et al.* (2003). During the subchronic study, male and female Crj:CD SD rats (5 to 6 weeks of age) were administered ϵ -polylysine at concentrations of 2,000, 10,000, and 50,000 ppm in the diet for 90 days (Ishii *et al.*, 1993). Reduction in body weight and corresponding reductions in organ weights were observed in the high dose males and females. These effects were associated with poor diet palatability. Mild histopathological changes in the liver also were reported in the high dose groups. Due to a number of statistically significant changes in various biochemical, hematological, and histopathological findings, which were limited to male and females consuming the high dose diets (50,000 ppm; 4,295 mg/kg/day), a no-observed-effect level of 10,000 ppm in the diet (895 mg/kg body weight in males and 995 mg/kg body weight in females) can be determined.

The chronic toxicity of ϵ -polylysine was evaluated using 320 Crj:CD SD rats (6 weeks of age) administered ϵ -polylysine in the diet at concentrations of 2,000, 6,500, and 20,000 ppm for up to 102 and 104 weeks respectively for male and females (Fukotome *et al.*, 1995).² No differences in animal survival between treatment groups in either sex were observed. No increases in the incidence of non-neoplastic or neoplastic lesions attributable to the test article were reported. Neither the liver or any other organ or tissue showed a treatment-related effect at any dose level following histopathological examinations. No consistent or dose-related alteration of clinical biochemistry, urinalysis or hematology was associated with treatment in the chronic feeding study. The only statistically significant finding reported by the authors was a transient reduction in weight gain during the mid-phase of the study in males, which recovered to control levels by 84 weeks. A reduction in weight gain, notably in male animals, is a consistent finding that has been reported in all the toxicology studies conducted with polylysine to date. Polylysine is well established to be bitter at high concentrations. During these studies, the observed reductions in weight gain were reported in animals consuming between 20,000 to 50,000 ppm in the diet, in all cases were limited to the highest dietary concentration with no evidence of dose responsive effects across large margins of food intakes, and were typically associated with corresponding reductions in food intake. These observations are consistent with the criteria highlighted by

¹ The articles were published in peer-reviewed Japanese journals, and contained English tables, figures, and abstracts. Additionally, English translations of these articles were provided to the Expert Panel.

² Study design was based on a 130-week treatment interval; however, due to animal mortality the experiment was terminated when the cumulative fatality ratio reached 75%.

Flamm *et al.*, (2003) as representing critical factors whereby reduced weight gain is attributed to palatability issues. This conclusion also is consistent with World Health Organization's (WHO) guidance (WHO, 1987) concerning the interpretation of lower body weight gain in the absence of other toxicity due to consumption of a test material with known nutritive and palatability effects. Thus, a NOAEL of 20,000 ppm in the diet or 1,060 mg/kg, which was the lower value ingested by the male rats (1,317 mg/kg in high dose females), can be determined. The previous Expert Panel, also considered a NOAEL of 20,000 ppm in the diet to be an appropriate determination for this study.

A two-generation reproduction and teratogenicity study conducted in Crj:CD SD rats³ administered ϵ -polylysine in the diet (0, 3,000, 10,000, or 30,000 ppm) was reported by Neda *et al.* (1999)⁴. No treatment related effects on reproductive, neurological, and immunological function, or embryonic and fetal development and growth were noted at any dose.

In vitro studies evaluating the mutagenicity of ϵ -polylysine were conducted using the bacterial reverse mutation assay in the presence and absence of metabolic activation using Ames tester strains *S. typhimurium* TA100, TA1535, TA98, and TA1537, as well as *E. coli* strain WP2 uvrA. Although the tests were limited to low doses due to the antimicrobial properties of the compound, no mutagenic effects were observed. These observations are consistent with the absence of neoplastic effects reported in the chronic toxicity evaluation.

The GRAS use of *S. albulus* subsp. lysinopolymerus, also was considered. It was determined *S. albulus* lysinopolymerus was GRAS for use in the synthesis of polylysine based on the following information: (1) Epsilon-polylysine is manufactured under cGMP employing suitable controls to prevent transfer of the fermentation organism to the final product (0.1 μ m filter sterilization, followed by several ion-exchange purification steps). (2) Microbial analyses of the purified ϵ -polylysine solution prior to spray drying, confirms that the fermentation strain is absent in the final product. (3) No evidence was identified in the literature indicating that members of *S. albulus* subsp. lysinopolymerus are potentially pathogenic or toxicogenic. (4) Studies demonstrating the absence of *Streptomyces* antibiotics in the product. (5) Finally, the safety of *S. albulus* subsp. lysinopolymerus for use in the production of a food grade ingredient also is supported by the absence of toxicity, mutagenicity, or carcinogenicity observed in the product specific rodent toxicology, and *in vitro* mutation studies.

Overall, the results of rodent toxicity studies and *in vitro* mutagenicity studies indicate that ϵ -polylysine is of low-toxicity and does not display genotoxic potential. Assuming a worst-case maximum exposure to polylysine from the proposed food uses, the daily total population all-user

³ Male rodents were between 208.8 to 263.5 g and females were between 145.7 to 176.6 g at the beginning of study. Housing and environmental conditions were as described in the subchronic and chronic studies.

⁴ Study was published in peer-reviewed Japanese journal, and contained English tables, figure, and abstract; English translation was not available.

intake was estimated to be 8.4 mg/kg body weight per day. Highest exposures were estimated for individual's aged 0 to 2 and 3 to 11, where 90th percentile all-user exposures were 14.8 and 13.0 mg/kg body weight/day respectively. The safety of the proposed uses of ϵ -polylysine in food is based on the significant margin of safety between the estimated exposures relative to the totality of evidence obtained from the available safety data. This conclusion was supported by the absence of toxicity reported in several rodent toxicity studies including the chronic administration of ϵ -polylysine to rats at a dose of up to 1,020 mg/kg body weight (the highest dose tested); that the 90th percentile intake estimates are considered gross over estimations of the actual exposures; that ϵ -polylysine is not absorbed and virtually no systemic exposure to the compound is expected when consumed in the diet under the proposed food uses. Thus, Purac concluded that provided ϵ -polylysine is manufactured in accordance with GMP, using suitable food grade ingredients, that the proposed expanded uses of ϵ -polylysine in food are GRAS based on scientific procedures.

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CONCLUSION

We, the Expert Panel, have, independently and collectively, critically evaluated the data and information summarized above and conclude that under the conditions of intended use and use levels as a preservative in certain selected conventional foods (when otherwise not precluded by Standards of Identity) as described in table A1, ϵ -polylysine, produced *via* a fermentation process involving *Streptomyces albulus* lysinopolymerus, meeting appropriate food-grade specifications, and manufactured and used in accordance with current good manufacturing practice, is safe for consumption and Generally Recognized as Safe (GRAS), based on scientific procedures. It is our opinion that other qualified experts would concur with these conclusions.

(b) (6)

December 9, 2009

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Cantox Health Sciences International

Date

(b) (6)

Stanley M. Tarka, Ph.D.
The Tarka Group, Inc.

10 December 2009

Date

(b) (6)

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Indiana University School of Medicine

14 December 2009

Date

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Attachment 1

Individual Proposed Food-Uses and Use-Levels for Polylysine in the U.S.

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Table A-1 Summary of the Individual Proposed Food-Uses and Use-Levels for Polylysine in the U.S.

Food Category	Proposed Food-Uses	Use-Levels (%)*
Alcoholic Beverages	Light Beer	0.01
	Wine	0.005
Baked Goods and Baking Mixes	Cake Batter and Cheesecakes	0.01
	Muffins and Popovers	0.01
	Pancakes and Waffles	0.025
	Pies (Not Fruit)	0.025
	Sweet Pastries	0.01 to 0.025
Beverages and Beverage Bases	Energy, Fitness, Sports, and Isotonic Drinks (RTD)	0.025
	Gelatin, Rice, and Sugar Cane-Based Beverages	0.025
	Soft Drinks	0.025
Cheeses	Cheese-Based Mixtures	0.025
	Cottage, Ricotta, and Cream Cheese	0.025
	Natural (Feta, Mozzarella, and Mexican)	0.025
	Processed Cheese and Cheese Spreads	0.025
Coffee and Tea	Coffee and Coffee Beverages	0.025
	Tea and Tea Beverages	0.025
Condiments and Relishes	Pickles, Olives, and Pickled Products	0.025
Dairy Product Analogs	Cream Substitutes	0.025
	Imitation Cheese	0.025
Egg Products	Egg-Based Dishes	0.025
Fats and Oils	Reduced-Fat or Fat-Free Margarine-like Spread	0.005
	Salad Dressings (Regular and Low-fat)	0.025
Fish Products	Fish and Shellfish-Based Foods	0.025
Fresh Eggs	Eggs	0.025
Fresh Fish	Fish and Shellfish	0.025
Fresh Meats	Beef (Steak, Stewing, and Ground)	0.06
	Pork (Chops and Steaks)	0.06
Fresh Poultry	Chicken Breast and Turkey	0.06
Fruit and Water Ices	Frozen Fruit Bars and Sorbet	0.025
Gelatins, Puddings, and Fillings	Gelatin	0.025
	Pie Fillings	0.025
	Puddings, Custards, and Mousses	0.025
Grain Products and Pastas	Grain-Based Dishes	0.025
	Pastas	0.025
Gravies and Sauces	Fat-Based Sauces	0.01
	Gravy	0.025
	Tomato-Based Sauces	0.025

Table A-1 Summary of the Individual Proposed Food-Uses and Use-Levels for Polylysine in the U.S.

Food Category	Proposed Food-Uses	Use-Levels (%)*
Meat Products	Meat-Based Prepared Foods	0.02 to 0.03
Milk and Milk Products	Chocolate-Flavored Drinks	0.01
	Cream	0.01
	Eggnog	0.01
	Sour Cream and Dips	0.025
	Yogurt	0.01
Nuts and Nut Products	Peanut Butters and Sauces	0.025
Plant Protein Products	Meat Substitutes	0.025
	Soy Products	0.025
Poultry Products	Poultry-Based Prepared Foods	0.025
Processed Fruits and Fruit Juices	Fruit-Flavored Drinks and Ades (RTD)	0.025
	Fruit Smoothies	0.025
	Nectars	0.025
	Prepared Fruit and Fruit Mixtures	0.025
Processed Vegetables and Vegetable Juices	Dry Beans and Bean-Based Prepared Foods	0.025
	Potato- and Sweet Potato-Based Foods (Excluding French Fries)	0.025
	Vegetable-Based Prepared Foods	0.01 to 0.025
	Tomato-Based Juices	0.025
Soups and Soup Mixes	Soups (Excluding Canned)	0.025
Sweet Sauces, Toppings, and Syrups	Sweet Syrups and Toppings	0.025

RTD = Ready-to-Drink.

* Use levels were based on 200 mg polylysine /RACC; RACC = Reference Amounts Customarily Consumed per Eating Occasion (21 CFR §101.12 – U.S. FDA, 2009b). When a range of values is reported for a proposed food-use, particular foods within that food-use may differ with respect to their RACC.

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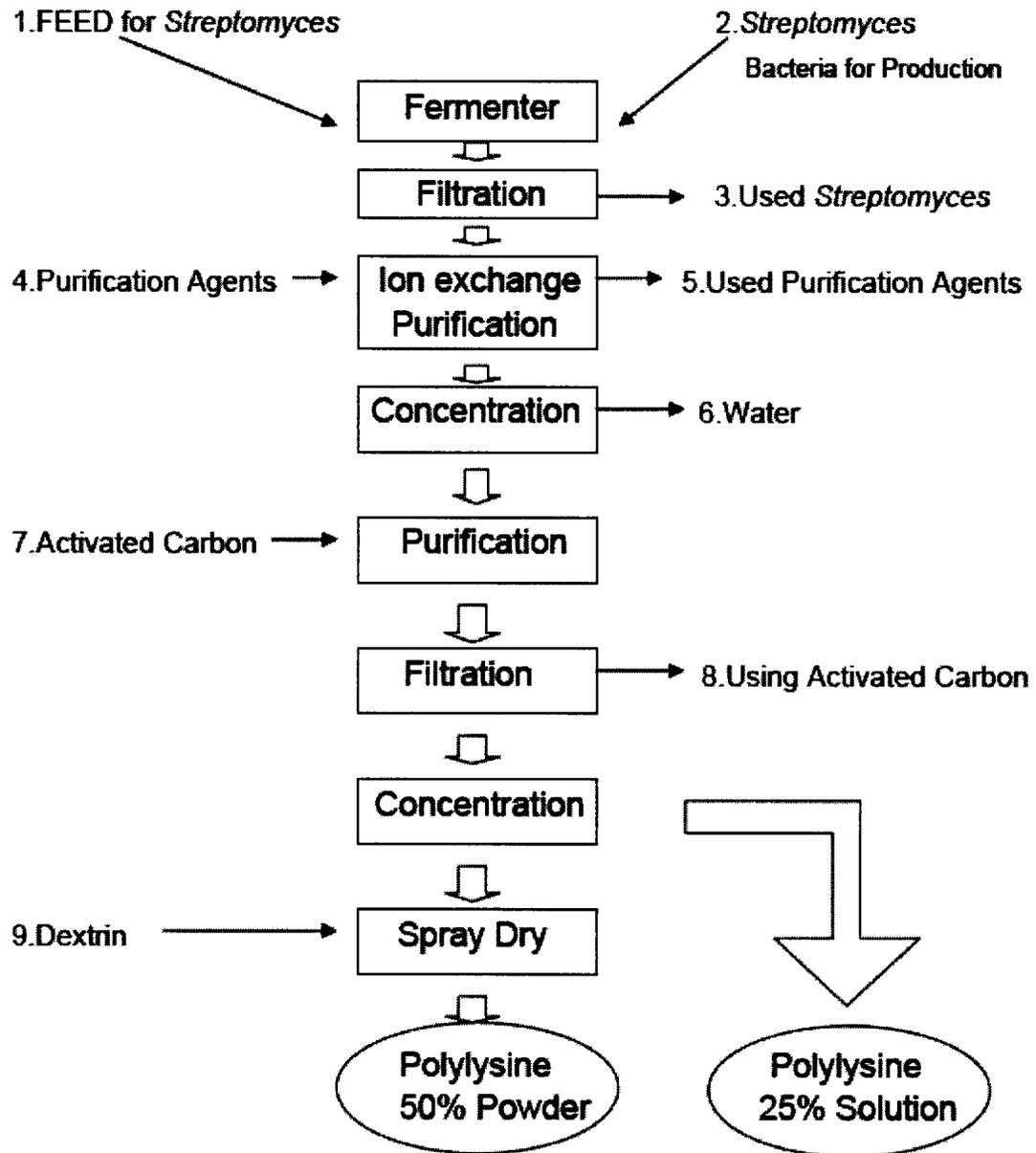
Attachment 2
Expert Panel *Curriculum vitae*

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Attachment 3
Manufacturing Overview and
Product Specifications

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Figure A3-1 Outline of Manufacturing Process for ϵ -Polylysine



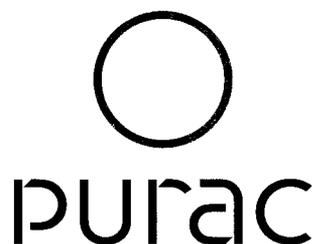
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TABLE A3-1 SPECIFICATION FOR PURAC FORMULATIONS: PURAQ XTEND FX50 P AND PURAQ XTEND FX25

Parameter	Specification		Analytical Methods
	PuraQ Xtend FX50P 50:50 Mixture of ϵ -Polylysine and Maltodextrose Powder	PuraQ Xtend FX25 ϵ -Polylysine 25% Solution in Water	
Purity	50.0 to 54.0%	25.0 to 27.0%	HPLC*
Heavy Metals (ppm)	≤ 10	≤ 10	ICP-OES
Lead (ppm)	< 1	≤ 1	ICP-OES
Total Aerobic count (cfu/g)	≤ 500	≤ 500	HGMF
Yeasts and Moulds (cfu/g)	≤ 500	≤ 500	HGMF

* In house validated method; HGMF = Hydrophobic Grid Membrane Filtration Method

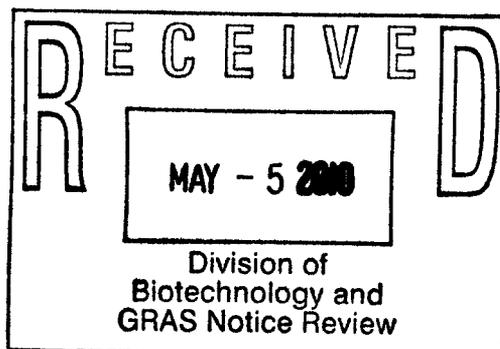
000060



SENT VIA FEDEX

April 05, 2010

Robert L. Martin, Ph.D.
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food And Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835



GRAS Notice for ϵ -Polylysine

Dear Dr. Martin:

In accordance with 21 CFR §170.36 [Notice of a claim for exemption based on a Generally Recognized As Safe (GRAS) determination] published in the *Federal Register* [62 FR 18938 (17 April 1997)], I am submitting in triplicate, as the Notifier [Purac Biochem b.v., Arkelsedijk 46 PO Box 21, 4206 AA Gorinchem, The Netherlands], a Notice of the determination, on the basis of scientific procedures, that ϵ -polylysine distributed by Purac, as defined in the enclosed documents, is GRAS under specific conditions of use as a preservative in various traditional foods, and therefore, is exempt from the premarket approval requirements of the *Federal, Food, Drug and Cosmetic Act*. Information setting forth the basis for the GRAS determination, includes a comprehensive summary of the data available that has been reviewed by an independent panel of experts (the Expert Panel) qualified by scientific training and experience to evaluate the safety of ϵ -polylysine in traditional food products. A fourth copy of this Notification also has been included for purview by the United States Department of Agriculture's Food Safety and Inspection Service regarding the uses of ϵ -polylysine as a preservative in meat and poultry, and meat and poultry containing products.

Should you have any questions or concerns regarding this GRAS Notice, please do not hesitate to contact me at any point during the review process so that we may provide a response in a timely manner.

Sincerely,

(b) (6)

Ton van Dongen
Global Regulatory Manager
Purac Biochem b.v.

000061

a  **CSM** company

SUBMISSION END

000062



Ramos-Valle, Moraima

From: Dongen, Ton van [T.vanDongen@purac.com]
Sent: Wednesday, April 28, 2010 2:38 AM
To: Ramos-Valle, Moraima
Cc: Farias, Bianca; Martin, Robert L; Wispelaere, Maureen de
Subject: RE: GRAS submission
Attachments: scannen0001.pdf

Dear Mrs. Ramos Valle,

Please find enclosed our cover letter without the "confidential" statement. I have kept the data the same as on the original letter.

I trust that this solves the matter.

In case of questions please feel free to contact me again.

Yours sincerely,

Ton van Dongen

From: Ramos-Valle, Moraima [mailto:Moraima.Ramos-Valle@fda.hhs.gov]
Sent: donderdag 22 april 2010 20:27
To: Dongen, Ton van
Cc: Farias, Bianca; Martin, Robert L
Subject: RE: GRAS submission

Dear Mr. Ton van Dongen,

Thanks for your clarification and confirmation that none of the data is confidential. We will appreciate if you could send us a revise letter without the confidential heading; we will need that in order to file your submission as a GRAS notice.

Please let me know if you have any questions.

Thanks,
Moraima

Moraima J. Ramos Valle
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review
Food and Drug Administration
Phone: 301-436-1248
Email: Moraima.Ramos-Valle@fda.hhs.gov

000063

From: Dongen, Ton van [mailto:T.vanDongen@purac.com]
Sent: Thursday, April 22, 2010 5:57 AM
To: Ramos-Valle, Moraima
Cc: Farias, Bianca; Martin, Robert L; Wispelaere, Maureen de
Subject: RE: GRAS submission

Dear Mrs. Ramos Valle,

Our GRAS submission mentioned below should not have had the confidential stamp.

I can confirm that all data filed by us in this GRAS notice are non-confidential.

Apologies for the confusion.

Yours sincerely,

Ton van Dongen
Purac Global Regulatory Manager

From: Ramos-Valle, Moraima [mailto:Moraima.Ramos-Valle@fda.hhs.gov]
Sent: dinsdag 20 april 2010 21:40
To: Dongen, Ton van
Cc: Farias, Bianca; Martin, Robert L
Subject: GRAS submission

Dear Mr. Ton van Dongen,

This message is to acknowledge the receipt of your GRAS submission dated April 12, 2010 and received by the Food and Drug Administration on April 16, 2010 for the use of epsilon-polylysine in foods. We noted that PURAC's cover letter has a confidential stamp. We are seeking clarification as whether this submission is confidential or not. As a reminder your submission has not been filed as a GRAS notice and we will not do any further review until we hear from you.

I will appreciate your prompt response and please feel free to contact me if you have any questions.

Sincerely,
Moraima

Moraima J. Ramos Valle
Consumer Safety Officer
Division of Biotechnology and GRAS Notice Review
Food and Drug Administration

000064

4/29/2010

Phone: 301-436-1248
Email: Moraima.Ramos-Valle@fda.hhs.gov

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The PURAC group of companies including its subsidiaries and/or its employees shall not be liable for the incorrect or incomplete transmission of this e-mail or any attachments, nor responsible for any delay in receipt;

*****;

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*****;

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SENT VIA FEDEX

April 05, 2010

Robert L. Martin, Ph.D.
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food And Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

GRAS Notice for ϵ -Polylysine

Dear Dr. Martin:

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Should you have any questions or concerns regarding this GRAS Notice, please do not hesitate to contact me at any point during the review process so that we may provide a response in a timely manner.

Sincerely,

(b) (6)

Ton van Dongen
Global Regulatory Manager
Purac Blochem b.v.

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