December 19, 2000

Office of Premarket Approval (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C St., SW
Washington, DC 20204

Re: GRAS Notice for Specified Uses of Egg White Lysozyme
     GRAS Notice for Specified Uses of Nisin
     GRAS Notice for Specified Uses of Hops Beta Acids

Dear Sir or Madame:

On behalf of my client, Rhodia, Inc., please accept the attached documentation, in compliance with the GRAS notification procedure set out in the April 17, 1997 Federal Register (62 FR 18937), as submissions of notices of GRAS exemption claims for the above referenced substances, i.e. specified uses of egg white lysozyme, specified uses of nisin, and specified uses of hops beta acids. As specified in the aforementioned proposed rule, each GRAS notice is submitted in triplicate and contains: a signed exemption claim; detailed information on the substance, on any self-limiting levels of use, and on the basis for the determination; and an appendix containing further referenced and substantiating information on the substance.

Please promptly contact me should you have any question regarding any of the submitted notices. We look forward to receiving acknowledgment of receipt of the notices and to a response for each noticed substance. Thank you.

Sincerely,

ROBERT H. SINDT

Enc.
RHS/bs
December 15, 2000

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

Re: GRAS Notice-Exemption Claim for Specified Uses of Egg White Lysozyme

Dear Dr. Kahl:

On behalf of my client, Rhodia Inc., and in accordance with FDA's proposed rule of April 17, 1997 (62 FR 18938) relating to the filing of generally recognized as safe (GRAS) notices, please accept this claim and the attached information, each submitted in triplicate, for that purpose as they relate to the use of egg white lysozyme in certain foods. Specifically, Rhodia claims that use of egg white lysozyme as an antimicrobial agent for frankfurters, and for cooked meat and poultry products sold ready-to-eat, is exempt from premarket approval requirements of the Federal Food, Drug and Cosmetic Act based on its determination that such use is GRAS. In conformity with the requirements outlined in the proposed rule, the following information is included with this exemption claim:

(i) Name and Address of the Notifier: Rhodia Inc.
   CN 7500
   259 Prospect Plains Road
   Cranbury, NJ 08512-7500

(ii) Common or Usual Name of Notified Substance: Egg white lysozyme, whose Chemical Abstract Service (CAS) registry number is 9001-63-2, and whose specifications conform to those for egg white lysozyme as set out by FDA in a tentative final rule published in the Federal Register on March 13, 1998 (63 FR 12421 et.seq.).

(iii) Applicable Conditions of Use: Egg white lysozyme is manufactured in compliance with current Good Manufacturing Practice as specified in 21 CFR Part 110 and the Food Chemicals Codex, Fourth Edition and any subsequent amendment thereto, and meeting the specifications for the substance set out in FDA's tentative final rule at 63 FR 12421 et.seq. Egg white lysozyme is
proposed for use as an antimicrobial agent in casings for frankfurters at a concentration of 2.5 mg egg white lysozyme/lb of frankfurter, approximately 5.5 mg egg white lysozyme/kg of food, and for use as an antimicrobial agent on cooked meat and poultry products sold ready-to-eat at 2.0 mg egg white lysozyme/lb of cooked meat or poultry product, approximately 4.4 mg egg white lysozyme/kg of food. All persons greater than two years of age are expected to consume the substance.

(iv) Basis for the GRAS Determination: Scientific procedures

(v) Availability to FDA of Data and Information that are Basis of Determination: The data and information forming the basis for Rhodia's GRAS determination and the exemption claim asserted herein are available for FDA review and copying during reasonable business hours at the following address, or will be sent to FDA upon request: Robert H. Sindt, Attorney at Law

Suite 400
1850 M Street, NW
Washington, DC 20036
Phone: (202) 466-4500

Consequently, on the basis of the above specified information, and the additional requested information as specified in the proposed rule and submitted with this letter, please accept this as Rhodia’s claim of exemption from the statutory premarket approval requirements for the use of egg white lysozyme as an antimicrobial agent for frankfurters and for cooked meat and poultry products sold ready-to-eat. Should you have any questions regarding the submission of this notice, please contact me at the above number. Thank you for your prompt consideration of, and response to, this notice.

Sincerely,

Robert H. Sindt

RHS:bs

Attachments

P:\Rhodia\Lysozyme GRAS Notice Claim.doc
LYSOZYME
GRAS NOTICE
INFORMATION

Rhodia
EGG WHITE LYSOZYME-GRAS NOTICE INFORMATION

(2) DETAILED INFORMATION ABOUT THE IDENTITY OF THE NOTIFIED SUBSTANCE (§170.36(c)(2))

- Common and Usual Name of the Food Grade Substance: Egg White Lysozyme

- Chemical Name for Lysozyme: The Commission on Enzymes of the International Union of Biochemistry's systematic name for lysozyme is peptidoglycan N-acetylmuramoylhydrolase and its systematic number is EC No. 3.2.1.17.

- Chemical Abstract Service (CAS) Registry Number for Lysozyme: 9001-63-2.

- Empirical Formula for Lysozyme: None

- Structural Formula for Lysozyme: In publishing a tentative final GRAS rule for egg white lysozyme (63 FR 12421 et.seq.), FDA noted at page 12422 that “Lysozyme was the first enzyme to have the detail of its three-dimensional structure published (Ref. 4), and it has become one of the best characterized of all enzymes, serving as an example for studies of enzyme mechanism and molecular evolution”. (Ref. 4 is Blake, C.C.F., D.F. Koenig, G. A. Mair, A. C. T. North, D.C. Phillips, and V. R. Sarma, “Structure of Hen Egg-White Lysozyme.” Nature. 206:757-783. 1965.’) FDA also went on note the similarity of lysozymes from different source organisms, noting that “Egg white lysozyme differs very little in structure, amino acid sequence
and composition, catalytic mechanism, and substrate specificity” from the enzyme found in several human fluids.

- Quantitative Composition for Lysozyme: Lysozyme is a natural antimicrobial protein made up of 129 amino acid residues, with a molecular weight of about 14,300 Da. While lysozyme is naturally occurring in many organisms, it is found in abundance in chicken egg white, the source of much egg white lysozyme that is isolated and purified for commercial food use. Egg white lysozyme conforms in all respects to the enzyme preparation described in FDA’s direct food substances affirmed as GRAS tentative final rule for the substance, published on March 13, 1998 (63 FR 12421 et.seq).

- Method of Manufacture for Egg White Lysozyme: Egg white lysozyme is manufactured in compliance with current Good Manufacturing Practice specified in 21 CFR, part 110, and in the Food Chemicals Codex, Fourth Edition and any subsequent amendment thereof.

  Methods used to isolate lysozyme from egg whites are based on available and accepted principles of protein purification. These methods do not alter the chemical identity or characteristic properties of the enzyme. Consequently, the quality, utility, safety, and functionality, including characteristic antibacterial activity, of the resultant egg white lysozyme are unchanged as a result of the isolation method.

  Lysozyme is extracted from fresh egg white, where it is present at approximately 0.3% of the egg white and 3.5% of the egg white protein. In the processing, a food-
grade inert material (a polymer resin) is mixed with egg white where it specifically binds with the lysozyme. The resin carrying the lysozyme is separated from the egg white. Then, the lysozyme is removed from the resin through the addition of salts and a change of pH. It is then concentrated, purified, and dried. The resulting purified protein on a dry basis is almost 100% lysozyme, although small amounts of other egg white proteins may be present.

Lysozyme produced from egg white conforms to the specification for “egg white lysozyme” set forth by the FDA in a direct food substances affirmed as generally recognized as safe tentative final rule published in the Federal Register on March 13, 1998 (63 FR 12421 et.seq.). It also complies with the general requirements and additional requirements for enzyme preparations of the Food Chemicals Codex, 4th ed.

- Characteristic Properties of Lysozyme: Lysozyme is a natural antimicrobial protein made up of 129-130 amino acid residues, having a molecular weight of about 14,300. Lysozyme is naturally occurring in many organisms, such as viruses, bacteria, plants, insects, birds, reptiles and mammals. In mammals, lysozyme has been isolated from nasal secretions, saliva, tears, intestines, urine and milk. Hen egg white lysozyme is among the most thoroughly characterized enzymes, and its three-dimensional structure, mechanism of action, substrate specificity, and other properties have been determined. Lysozyme is inactivated by stomach and intestinal proteolytic enzymes, particularly pepsin, rendering it harmless to humans on ingestion.
The most common source of food grade lysozyme is from chicken egg whites. Lysozyme is an enzyme that has been shown to possess antimicrobial properties, especially in relation to *Clostridium tyrobutyricum*. Egg white lysozyme is the common and usual name. The Commission on Enzymes of the International Union of Biochemistry's system for naming enzymes assigns both a name and number to each enzyme. The systematic name of lysozyme is peptidoglycan N-acetylmuramoylhydrolase and the systematic number is EC No. 3.2.1.17. The Chemical Abstract Service (CAS) registry number is 9001-63-2.

- Content of Potential Human Toxicants for Lysozyme: None.

- Specifications for Food Grade Egg White Lysozyme: Egg white lysozyme conforms in all respects to the specifications and characteristics set forth by FDA in its tentative final rule for the substance, published on March 13, 1998 (63 FR 12421 et.seq.), including the general requirements and additional requirements for enzyme preparations of the Food Chemicals Codex, 4th ed. Technical specifications for a typical food grade commercial egg white lysozyme, lysozyme hydrochloride (where HCl salts were utilized in removing the lysozyme from the egg whites), follow:

(Muramidase, Mucopeptide N-acetylmuramoylhydrolase; E.C. No. 3.2.1.17)

<table>
<thead>
<tr>
<th>Source:</th>
<th>Chicken egg white</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance:</td>
<td>Dialyzed, white to off-white powder</td>
</tr>
<tr>
<td>Ash:</td>
<td>Max. 2.0%</td>
</tr>
<tr>
<td>Moisture:</td>
<td>Max. 6.0%</td>
</tr>
<tr>
<td>Property</td>
<td>Specification</td>
</tr>
<tr>
<td>--------------------------</td>
<td>---------------------------------------------------</td>
</tr>
<tr>
<td>pH</td>
<td>-3.5 - 4.5</td>
</tr>
<tr>
<td>Solubility</td>
<td>-Min. 95% T. (1.5% Solution OD 640nm)</td>
</tr>
<tr>
<td>Bulk Density</td>
<td>-Minimum 0.5g/ml</td>
</tr>
<tr>
<td>Chloride</td>
<td>-Max. 3.5%</td>
</tr>
<tr>
<td>Heavy Metals</td>
<td>-Less than 10ppm</td>
</tr>
<tr>
<td>Arsenic</td>
<td>-Less than 2ppm</td>
</tr>
<tr>
<td>Lead</td>
<td>-Less than 2ppm</td>
</tr>
<tr>
<td>Standard Plate Count</td>
<td>-Less than 100/gram</td>
</tr>
<tr>
<td>Yeast/Mold</td>
<td>-Less than 100/gram</td>
</tr>
<tr>
<td>Storage</td>
<td>-Original package and at cool temperature</td>
</tr>
<tr>
<td>Activity</td>
<td>-Min. 95% (min 20,000 Shugar units/mg)</td>
</tr>
</tbody>
</table>
EGG WHITE LYSOZYME-GRAS NOTICE INFORMATION

(3) INFORMATION ON SELF-LIMITING LEVELS OF USE. IF ANY

(§170.36(c)(3))

- No information on self limiting levels of egg white lysozyme use is noted.
EGG WHITE LYSOZYME-GRAS NOTICE INFORMATION

(4) DETAILED SUMMARY OF THE BASIS FOR GRAS DETERMINATION (§170.36(c)(4))

Rhodia's determination, that the notified uses of egg white lysozyme (as an antimicrobial agent on casings for frankfurters and for cooked meat and poultry products sold ready-to-eat) are exempt from premarket approval requirements because such uses are GRAS, is based on scientific procedures. The determination was confirmed by an independent panel of scientific experts convened by Rhodia to conduct such a critical review. Each member of the independent expert panel was qualified by their scientific training and experience to evaluate the safety of substances used in food. The independent expert panel's report and determination, dated October 2000, is included in its entirety in the Appendix attached hereto.

(A) Safety of Egg White Lysozyme:

Egg white lysozyme, for the uses proposed herein, meets the specifications for the substance as set out by FDA in the direct food substances affirmed as GRAS tentative final rule, published on March 13, 1998 (63 FR 12421 et.seq.) for use in cheese manufacture as an antimicrobial substance. In publishing the tentative final rule, FDA engaged in an extensive discussion of the petition filed asserting GRAS status, and the discovery, history of food use, safety, exposure, sources, manufacturing methods, characteristics, toxicity, antibiotic resistance, labeling, and other relevant issues regarding egg white lysozyme. FDA concluded, without question, that egg white lysozyme was GRAS, but left the
final rule tentative due to its desire to receive data and comments on the necessity for certain labeling of the substance based on possible allergenicity concerns. Indeed, the tentative final rule contained, on page 12425, an extensive reference listing of published scientific data and information pertinent to both safety and common knowledge documentation in reaching its GRAS conclusion. A copy of the tentative final rule, as published, is contained in the Appendix attached hereto.

The independent expert panel convened by Rhodia also referred to the published data and information cited by FDA, and, in its report, the panel stated “There are extensive data, including numerous published and unpublished data and reports, to support the safety of lysozyme in foods. The studies are cited in the dossier (Appendix 2). Such studies and information were also evaluated and thoroughly discussed by FDA in publishing the aforementioned tentative final rule on egg white lysozyme. The safety of Lysozyme as a component of egg white is also supported by a long history of safe consumption by humans as a source of food protein throughout recorded history.”

In connection with its determination, Rhodia requested Novigen Sciences, Inc. (Novigen) to conduct an estimated dietary intake assessment for the proposed uses of egg white lysozyme. The complete intake assessment from Novigen is included in the Appendix attached hereto. Of particular significance are the following passages from the Novigen assessment: "At the request of Rhodia, Inc. (Rhodia), Novigen Sciences, Inc. (Novigen) has estimated the dietary intake of lysozyme by the U.S. population and selected population subgroups in support of a self-assessment of GRAS status. Lysozyme is proposed for use in casings for
frankfurters at concentrations of 2.5 mg lysozyme/lb frankfurter (approximately 5.5 mg lysozyme/kg food) and in cooked meats sold ready-to-eat at 2.0 mg lysozyme/lb cooked meat (approximately 4.4 mg lysozyme/kg food).

"Intakes were estimated from food consumption data collected by the U.S. Department of Agriculture (USDA). For the overall U.S. population, 2-day average per-user intakes ranged from 0.21 mg/person/day at the mean to 0.52 mg/person/day at the 95th percentile of intake. On a per-capita basis, intakes for the U.S. population ranged from 0.09 mg/person/day at the mean to 0.38 mg/person/day at the 95th percentile.

"Intake estimates based on individual survey days (i.e., not two-day average) ranged from 0.09 mg/person/day (mean per-capita) to 0.50 mg/person/day at the 95th percentile per-capita intake. On a per-user basis, intake estimates ranged from a mean of 0.34 mg/person/day to 0.81 mg/person/day at the 95th percentile of intake."

The independent expert panel convened by Rhodia also reviewed the Novigen intake assessment report, and further observed that "The Expert Panel also noted and concurred with FDA's conclusion, stated in the above referenced tentative final rule on egg white lysozyme (63 FR at 12422), that "since the egg whites from which lysozyme is extracted will be subsequently consumed in other food uses, there will be no longterm increase in lysozyme intake by the general population because egg whites without lysozyme will replace egg whites in current use that contain lysozyme."

LYSOZYME-GRAS NOTICE EXEMPTION CLAIM
Finally, the independent expert panel convened by Rhocia provided pertinent background and support for its safety finding by observing in its report that "Lysozyme was first discovered in 1922, being identified as an antibacterial enzyme present in nasal mucus membranes of humans. Several classes of lysozyme have been identified in nature, such as type C (chicken) lysozyme from domestic laying hens, type G after the Emden goose, human lysozyme, V types from certain bacteriophages, and H types from certain plants. Because lysozyme is abundant in the albumen of domestic hen's eggs, egg albumen has been the traditional source for use in foods. Egg white is a GRAS substance. The antimicrobial activity of lysozyme is related to its ability to catalyze the hydrolysis of the structural polysaccharide peptidoglycan found in the cell walls of bacteria. Resistant mutants have not been shown to arise even after continued treatment. Lysozymes are present in bacteria, fungi, plants and animal tissues; high concentrations are found in milk, saliva, mucus, and tears, and in egg white of domestic laying hens. Lysozyme is directly added to foods as the hydrochloride salt to specifically inactivate or inhibit spoilage and to protect against pathogenic bacteria. In particular, lysozyme is an effective inhibitor of the outgrowth of Clostridium tyrobutyricum to minimize "late blowing" in cheese manufacturing. It does not have lytic activity against beneficial lactic acid bacteria used in cheese fermentation. This use in certain cheese varieties has been so noted by the U.S. Food and Drug Administration (FDA) in publishing a tentative final rule for direct food substances affirmed as generally recognized as safe (63 FR 12421 et.seq.). Current good manufacturing practice allows use of a
level to inhibit or inactivate certain microorganisms, particularly *Clostridium
tyrobutyricum*. It is further noted that lysozyme has also been accepted by the
Joint WHO/FAO Expert Committee on Food Additives (JECFA) and the
Scientific Committee for Foods of the Commission of the European
Communities, and has been approved by Federation Internationale de Laiterie-
International Dairy Federation and by France and other European countries for
food uses. Also, egg white, the source of the lysozyme under review, has been
safely consumed by humans for as long as history has been recorded.”

(B) Information that may Appear Inconsistent with GRAS Determination:

In making its GRAS determination, Rhodia’s independent expert panel stated
that “No information on lysozyme is noted that appears to be inconsistent with the
determination of safety or general recognition of safety for the proposed uses.”

In reaching its conclusion during its critical evaluation, the independent
expert panel noted in its report that “The Expert Panel notes, and concurs with
FDA’s conclusion stated in the referenced tentative final rule, that proteins
derived from egg whites do not raise toxicity concerns and that the methods used
for extracting lysozyme from the egg white source should not alter either the
chemical identity or the characteristic properties of the enzyme. The Expert Panel
also concurs with FDA that there is insufficient current information to establish
whether the ingestion of egg white derived lysozyme elicits an allergenic response
when consumed by sensitive individuals. Therefore, the Expert Panel does not
address the labeling issue posed by FDA in its GRAS affirmation, considering the same to be beyond the scope of its review.

(C) Expert Concensus for GRAS Determination:

In publishing its tentative final rule on the GRAS status of egg white lysozyme, FDA cited numerous published data and information supporting its finding of concensus of common knowledge among qualified experts in the scientific community of the safety of, and lack of harm under the conditions of food use of, egg white lysozyme. Both Rhodia, and the independent panel of scientific experts it convened, concurred with FDA's assessment and also determined the same to be true for the uses proposed herein. Specifically, the independent expert panel noted in its report that "There are extensive data, including numerous published and unpublished data and reports, to support the safety of lysozyme in foods. The studies are cited in the dossier (Appendix 2). Such studies and information were also evaluated and thoroughly discussed by FDA in publishing the aforementioned tentative final rule on egg white lysozyme. The safety of Lysozyme as a component of egg white is also supported by a long history of safe consumption by humans as a source of food protein throughout recorded history."

Based on the information contained in the exemption claim, the above additional and supplementary information, and the information contained in the Appendix attached hereto, an ample basis exists to support determination of general recognition of safety for the meat and poultry uses of egg white lysozyme...
proposed herein. Indeed, the independent expert panel indicated a consensus of common knowledge of safety of the proposed uses of lysozyme among the qualified scientific community in concluding its review by determining that "The members of the Expert Panel, having independently and collectively critically evaluated the information summarized above and included in the appendices to this report, unanimously conclude that lysozyme, when produced in accordance with current Good Manufacturing Practice and meeting appropriate food grade specifications, is safe for use as an antimicrobial agent in certain foods. This conclusion is consistent with the opinion of the FDA that lysozyme is safe for use as an antimicrobial agent in certain foods.

"The members of the Expert Panel further concluded, based upon general availability of relevant information summarized herein which provides a basis upon which to find a consensus exists among qualified experts relating to safety, that lysozyme, produced in accordance with current Good Manufacturing Practice and meeting appropriate food grade specifications, is generally recognized as safe (GRAS) based on scientific procedures for use as an antimicrobial agent on cooked meat and poultry products as specified herein."
LYSOZYME
SELF GRAS
DETERMINATION

APPENDIX

Rhodia
LYSOZYME GRAS NOTICE APPENDIX

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<td>General Lysozyme Information</td>
<td>000024 to 000031</td>
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INDEPENDENT GENERALLY RECOGNIZED AS SAFE DETERMINATION OF

LYSOZYME

(IN CERTAIN COOKED MEAT AND POULTRY PRODUCTS)

OCTOBER 2000
INDEPENDENT GRAS DETERMINATION OF LYSOZYME

1. Introduction
The undersigned, an independent panel of recognized experts (hereinafter, the Expert Panel), qualified by their scientific training and relevant national and international experience in evaluating the safety of food and food ingredients, were requested by Rhodia, Inc to determine the generally recognized as safe (GRAS) status of hen egg white lysozyme for use as an antimicrobial agent in certain cooked meat and poultry products as further specified herein. The members of the Expert Panel include Professor Joseph F. Borzelleca (Medical College of Virginia), Professor Eric A. Johnson (University of Wisconsin), and John Cerveny (formerly Director of Product Safety at Oscar Mayer). The qualifications of the members of the Expert Panel are evidenced in their curricula vitae which appear in Appendix 1.

2. Basis for GRAS Status
Rhodia, Inc conducted a comprehensive search of the scientific literature for safety, toxicity, efficacy, and tolerance on lysozyme through 30 June 2000 and made this available to the Expert Panel. In addition, Rhodia, Inc provided the Expert Panel with information and data on the chemical, physical, and antimicrobial properties, manufacture and processing, stability, conditions of anticipated use, estimated daily intakes resulting from these uses, and safety of lysozyme. This information was consolidated by Rhodia in a document attached as Appendix 2 (the dossier). The Expert Panel independently and critically evaluated such information and data and other materials deemed appropriate or necessary, conferred by telephone, and then met in Chicago (26/27 July 2000) with technical representatives of Rhodia, Inc. and Viskase Corporation and other technical experts. The Expert Panel then critically evaluated all the available information and unanimously concluded that lysozyme, manufactured in accordance with current Good Manufacturing Practice (GMP) and meeting appropriate food grade specifications, is GRAS by scientific procedures for use as an antimicrobial agent in certain cooked meat and poultry products as specified herein at levels not to exceed current GMP.

3. History of Use
Lysozyme was first discovered in 1922, being identified as an antibacterial enzyme present in nasal mucus membranes of humans. Several classes of lysozyme have been identified in nature, such as type C (chicken) lysozyme from domestic laying hens, type G after the Emden goose, human lysozyme, V types from certain bacteriophages, and H types from certain plants. Because lysozyme is abundant in the albumen of domestic hen’s eggs, egg albumen has been the traditional source for use in foods. Egg white is a GRAS substance. The antimicrobial activity of lysozyme is related to its ability to catalyze the hydrolysis of the structural polysaccharide peptidoglycan found in the cell walls of bacteria. Resistant mutants have not been shown to arise even after continued treatment. Lysozymes are present in bacteria, fungi, plants and animal tissues; high concentrations are found in milk, saliva, mucus, and tears, and in egg white of domestic laying hens. Lysozyme is directly added to foods as the hydrochloride salt to specifically inactivate or inhibit spoilage and

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pathogenic bacteria. In particular, lysozyme is an effective inhibitor of the outgrowth of *Clostridium tyrobutyricum* to minimize “late blowing” in cheese manufacturing. It does not have lytic activity against beneficial lactic acid bacteria used in cheese fermentation. This use in certain cheese varieties has been so noted by the U.S. Food and Drug Administration (FDA) in publishing a tentative final rule for direct food substances affirmed as generally recognized as safe (63 FR 12421 et seq.). Current good manufacturing practice allows use of a level to inhibit or inactivate certain microorganisms, particularly *Clostridium tyrobutyricum*. It is further noted that lysozyme has also been accepted by the Joint WHO/FAO Expert Committee on Food Additives (JECFA) and the Scientific Committee for Foods of the Commission of the European Communities, and has been approved by Federation Internationale de Laiterie-International Dairy Federation and by France and other European countries for food uses. Also, egg white, the source of the lysozyme under review, has been safely consumed by humans for as long as history has been recorded.

4. Characteristics of Lysozyme

Egg white lysozyme is a natural antimicrobial protein made up of 129 amino acid residues, having a molecular weight of about 14,300 Da. Lysozyme occurs naturally in many organisms, such as viruses, bacteria, plants, insects, birds, reptiles and mammals. In mammals, lysozyme has been isolated from nasal secretions, saliva, tears, intestines, urine and milk. Hen egg white lysozyme is among the most thoroughly characterized enzymes, and its three-dimensional structure, mechanism of action, substrate specificity, and other properties have been determined. Lysozyme is inactivated by stomach and intestinal proteolytic enzymes, particularly pepsin, rendering it harmless to humans on ingestion. The most common source of food grade lysozyme is from chicken egg whites. Lysozyme is an enzyme that has been shown to possess antimicrobial properties, especially in relation to *Clostridium tyrobutyricum*. Lysozyme is the common and usual name. The hydrochloride salt of lysozyme is the form generally used in foods. The Commission on Enzymes of the International Union of Biochemistry’s system for naming enzymes assigns both a name and number to each enzyme. The systematic name of lysozyme is peptidoglycan- N-acetylmuramoylhydrolase and the systematic number is EC No 3.2.1.17 The Chemical Abstract Service (CAS) registry number is 90001-63-2

5. Manufacture

Lysozyme is manufactured in compliance with current Good Manufacturing Practice specified in 21 CFR, part 110, the Food Chemical Codex, Fourth Edition and any subsequent amendment thereof. Methods used to isolate lysozyme from egg whites are based on available and accepted principles of protein purification which do not alter the chemical identity or characteristic properties of the enzyme. Therefore, the quality, utility, safety, and functionality, including characteristic antibacterial activity, of lysozyme are unchanged as a result of the isolation method.

Lysozyme is extracted from egg white, where it is present at approximately 0.3% of the egg white and 3.5% of the egg white protein. In processing, a food-grade polymer resin is mixed with egg white and specifically binds lysozyme. The resin carrying the lysozyme is
then separated from the egg white. The lysozyme is removed from the resin by addition of salts and change of pH, and then concentrated, purified, and dried. Thus, the isolated enzyme for food use is the water-soluble hydrochloride salt. The resulting purified protein on a dry basis is almost 100% lysozyme hydrochloride, although small amounts of other egg white proteins may be present.

Lysozyme produced from egg white conforms to the specification for ‘egg white lysozyme’ set forth by the FDA in a direct food substances affirmed as generally recognized as safe tentative final rule published in the Federal Register on March 13, 1998 (63 FR 12421). It also complies with the general requirements and additional requirements for enzyme preparations of the Food Chemicals Codex, 4th ed. Technical specifications for lysozyme are set out in detail in Appendix 2.

6. Uses
Lysozyme is proposed for use in casings for frankfurters at concentrations of 2.5 mg lysozyme/lb frankfurter or approximately 5.5 mg lysozyme/kg food, and in cooked meat and poultry products sold ready-to-eat at 2.0 mg lysozyme/lb of cooked meat or poultry product or approximately 4.4 mg lysozyme/kg food (as specified in Exposure Assessment, Appendix 2).

No information on self-limiting levels of use was noted.

7. Exposure
Intakes were estimated from food consumption data collected by the U.S. Department of Agriculture (USDA). For the overall U.S. population, 2-day average per-user intakes ranged from 0.21 mg/person/day at the mean to 0.52 mg/person/day at the 95th percentile of intake. On a per-capita basis, intakes for the U.S. population ranged from 0.09 mg/person/day at the mean to 0.38 mg/person/day at the 95th percentile.

Intake estimates based on individual survey days (i.e., not two-day average) ranged from 0.09 mg/person/day (mean per-capita) to 0.50 mg/person/day at the 95th percentile per-capita intake. On a per-user basis, intake estimates ranged from a mean of 0.34 mg/person/day to 0.81 mg/person/day at the 95th percentile of intake.

The Expert Panel also noted and concurred with FDA’s conclusion, stated in the above referenced tentative final rule on egg white lysozyme (63 FR at 12422), that since the egg whites from which lysozyme is extracted will be subsequently consumed in other food uses, there will be no longterm increase in lysozyme intake by the general population because egg whites without lysozyme will replace egg whites in current use that contain lysozyme.
8. Safety
The FDA (1998) critically evaluated available information on lysozyme and affirmed its
GRAS status in a tentative final rule. The FDA requested public comment on the necessity
of including certain labeling requirements relating to allergenicity concerns.

There are extensive data, including numerous published and unpublished data and reports,
to support the safety of lysozyme in foods. The studies are cited in the dossier (Appendix
2). Such studies and information were also evaluated and thoroughly discussed by FDA in
publishing the aforementioned tentative final rule on egg white lysozyme. The safety of
lysozyme as a component of egg white is also supported by a long history of safe
consumption by humans as a source of food protein throughout recorded history.

The Expert Panel notes, and concurs with FDA's conclusion stated in the referenced
tentative final rule, that proteins derived from egg whites do not raise toxicity concerns
and that the methods used for extracting lysozyme from the egg white source should not
alter either the chemical identity or the characteristic properties of the enzyme. The
Expert Panel also concurs with FDA that there is insufficient current information to
establish whether the ingestion of egg white-derived lysozyme elicits an allergenic
response when consumed by sensitive individuals. Therefore, the Expert Panel does not
address the labeling issue posed by FDA in its GRAS affirmation, considering the same to
be beyond the scope of its review.

No information on lysozyme is noted that appears to be inconsistent with the
determination of safety or general recognition of safety for the proposed uses.
Summary and Conclusions

The members of the Expert Panel, having independently and collectively critically evaluated the information summarized above and included in the appendices to this report, unanimously conclude that lysozyme, when produced in accordance with current Good Manufacturing Practice and meeting appropriate food grade specifications, is safe for use as an antimicrobial agent in certain foods. This conclusion is consistent with the opinion of the FDA that lysozyme is safe for use as an antimicrobial agent in certain foods.

The members of the Expert Panel further concluded, based upon general availability of relevant information summarized herein which provides a basis upon which to find a consensus exists among qualified experts relating to safety, that lysozyme, produced in accordance with current Good Manufacturing Practice and meeting appropriate food grade specifications, is generally recognized as safe (GRAS) based on scientific procedures for use as an antimicrobial agent on cooked meat and poultry products as specified herein.

Joseph D. Borchardt, Ph.D.
Professor, Pharmacology & Toxicology
Medical College of Virginia

John G. Cerveny
Consultant, Microbiology and Food Safety

Eric A. Johnson, Sc.D.
Professor, Microbiology
University of Wisconsin
LYSOZYME
SELF GRAS
DETERMINATION
NOVAGARD™ (lysozyme)

Description and Nomenclature:

Lysozyme is a natural antimicrobial protein made up of 129-130 amino acid residues, with a molecular weight of about 14,400 (Hen egg white lysozyme). Lysozyme is naturally occurring in many organisms such as viruses, bacteria, plants, insects, birds, reptiles and mammals. In mammals, lysozyme has been isolated from nasal secretions, saliva, tears, intestines, urine and milk. One of the sources of lysozyme that is most abundant is chicken egg white. It is egg white lysozyme that is isolated and purified by Canadian Inovatech Inc. It is this lysozyme that is toll-manufactured and packaged for Rhodia under the name NovaGARD.

Appendix ___ lists the current approvals for Inovatech’s (Inovapure’s) egg white lysozyme.

A.  Common or Usual Name:

Lysozyme

H.  Chemical Name:

Muramidase, & Mucopeptide N-acetylmuramoylhydrolase

C.  Chemical Abstract Service (CAS) registry number:

9001-63-2
D. Empirical Formula:


E. Structural Formulas:


F. Specifications for food grade material:

Attached, as Appendix _ are the Technical Specifications and a sample Certificate of Analysis for lysozyme chloride currently being manufactured and packaged for Rhodia, Inc. by Canadian Inovatech, Abbotsford, British Columbia. All product is manufactured according to 21 CFR, part 110 for Good Manufacturing Practices.

G. Quantitative compositions:

Formula and Product Label:

Egg white lysozyme

H. Manufacturing Process:

Lysozyme is extracted from fresh egg white by means of a unique biotechnological process, developed by Inovatech. A food-grade inert material (a polymer resin) is mixed with the egg white where it binds specifically with the lysozyme. The resin carrying the lysozyme is separated from the egg white. The lysozyme is then stripped off the resin, concentrated, purified and dried. The content of lysozyme in egg white is approximately 0.3% and when it is removed using this process, the whites remain virtually unaffected. The purified protein on a dry basis, is almost 100% lysozyme.
Hen egg white lysozyme is relatively stable in cheese milk and whey obtained by acid precipitation or rennet curdling. It is packed in an airtight package and stored at room temperature. It retains activity for at least one year.
FLOW CHART FOR LYSOZYME PROCESSING

EGG WHITE
Run through ion exchange column

Ion exchange column eluted with buffer/salt solution

Lysozyme removed
Egg White

Lysozyme in buffer/salt solution
1. precipitated by cooling
2. Filtration

Redissolve crude lysozyme ppt

Filtration

Buffer/salt solution rejected

Foreign proteins rejected

Lysozyme and impurities (salt and water)

Ultrafiltration or Electro-Dialysis

Salt

Lysozyme and water

Spray-dry

Water

Powdered Lysozyme

Powder to Reprocess

Sifter

Quality Control

Powder to Reprocess

Lysozyme to be packaged and shipped
APPROVAL OF INOVAPURE'S USE AS A FOOD ADDITIVE

APPROVAL BY MINITERE DE L'AGRICOLTURE - Direction de la Qualite
Bureau PE/CC ref. 81408/RF, Paris, France 22/5/1981.

APPROVAL BY FIL-IDF (Federation Internationale de Laiterie International Dairy Federation)

TENTATIVE RULE BY FDA AS GRAS March 13, 1998
63 Federal Register 12421 12426

ACCEPTANCE BY SCIENTIFIC COMMITTEE FOR FOODS OF THE COMMISSION OF THE EUROPEAN COMMUNITIES.
Brussel 28/11/1991 - 013620/n/C1L/01/10/09/JH/pb

ACCEPTANCE BY JEFCA (Joint Expert Committee on Food Additives of Codex Alimentarius) of FAO/WHO.
TECHNICAL SPECIFICATIONS

LYSOZYME CHLORIDE

(Muramidase, Mucopeptide N-acetylmuramoylhydrolase; E.C. No. 3.2.1.17)

Type II: Food Grade

- Chicken Egg White
- Dialyzed, white to off-white powder

Source:

Appearance:

Ash:

- Max. 2.0%

Moisture:

- Max. 6.0%

pH:

- 3.5 - 4.5

Solubility:

- Min. 95% T. (1.5% Solution OD 640 nm)

Bulk Density:

- Minimum 0.5g/ml

Chloride:

- Max. 3.5%

Heavy Metals:

- Less than 10ppm

Arsenic:

- Less than 2ppm

Standard Plate Count:

- Less than 100/gram

Yeast/Mold:

- Less than 10/gram

Storage:

- Original package and at cool temperature

Activity:

- Min. 95% (min. 20,000 Shugar units/mg)

Activity determined by two procedures:

1. Units/mg Solid = \( \frac{A_{450} \text{ Minute}}{0.001 \times \text{mg solid/Reaction Mixture}} \)

   Unit Definition:
   One Unit is the amount of enzyme which causes a decrease of absorbance of 0.001 per minute at 450nm and pH 6.2 at 25°C.

2. Compared to Standard of The National Institute of Hygienic Sciences where 1mg of Standard contains 1mg lysozyme (potency), after drying.
(SAMPLE)

CERTIFICATE OF ANALYSIS

FOOD GRADE LYSOZYME CHLORIDE (POWDER)

(*Muramidase, Mucoprotein N-acetylmuramoylhydrolase: E.C. No. 3.2.1.17*)

Lot #: **A6281**

Product Code: **A**

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<thead>
<tr>
<th>SOURCE</th>
<th>APPEARANCE</th>
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<tbody>
<tr>
<td></td>
<td>- Chicken egg white</td>
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<tr>
<td></td>
<td>- Dialyzed, white amorphous powder</td>
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<table>
<thead>
<tr>
<th>ASH</th>
<th>MOISTURE</th>
</tr>
</thead>
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<tr>
<td></td>
<td>- Max. 2.0%</td>
</tr>
<tr>
<td></td>
<td>- Max. 6.0%</td>
</tr>
<tr>
<td></td>
<td>- 3.5 - 4.5</td>
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<table>
<thead>
<tr>
<th>SOLUBILITY</th>
<th>BULK DENSITY</th>
<th>CHLORIDE</th>
<th>STANDARD PLATE COUNT</th>
<th>YEAST/MOLD</th>
<th>STORAGE</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>- Min. 95% T (1.5% solution OD&lt;sub&gt;550&lt;/sub&gt;nm)</td>
<td>- Min. 0.5g/ml</td>
<td>- Max. 3.5%</td>
<td>- Less than 100/gram</td>
<td>- Original package and at cool temperature</td>
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<tr>
<td></td>
<td>99.7%</td>
<td>0.5</td>
<td>test passed</td>
<td>&lt;100/g</td>
<td>98%</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;10/g</td>
<td>23,800</td>
</tr>
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</table>

Activity determined by two procedures:

1. Units/mg Solid = \( \frac{A_{sg/Minute}}{0.001 \times mg\ solid/Reaction\ Mixture} \)

Unit Definition:

One unit is the amount of enzyme which causes a decrease of absorbance of 0.001 per minute at 450 nm and pH 6.2 at 25°C.

2. Compared to standard of The National Institute of Hygienic Science where 1 mg of Standard contains 1 mg Lysozyme (potency), after drying.

DATED: July 10, 2000            Quality Control

000059
ESTIMATED DIETARY INTAKE OF LYSOZYME
AS PROPOSED FOR USE IN FOODS IN THE US

PREPARED FOR:
Rhodia, Inc.
CN7500 Prospect Plains Road
Cranbury, NJ 08512-7500

PREPARED BY:
Novigen Sciences, Inc.
1730 Rhode Island Avenue, NW
Washington, DC 20036

July 11, 2000
I. INTRODUCTION

At the request of Rhodia, Inc. (Rhodia), Novigen Sciences, Inc. (Novigen) has estimated the dietary intake of lysozyme by the U.S. population and selected population subgroups in support of a self-assessment of GRAS status. Lysozyme is proposed for use in casings for frankfurters at concentrations of 2.5 mg lysozyme/lb frankfurter (approximately 5.5 mg lysozyme/kg food) and in cooked meats sold ready-to-eat at 2.0 mg lysozyme/lb cooked meat (approximately 4.4 mg/kg food).

Intakes were estimated from food consumption data collected by the U.S. Department of Agriculture (USDA). For the overall U.S. population, 2-day average per-user intakes ranged from 0.21 mg/person/day at the mean to 0.52 mg/person/day at the 95th percentile of intake. On a per-capita basis, intakes for the U.S. population ranged from 0.09 mg/person/day at the mean to 0.38 mg/person/day at the 95th percentile.

Intake estimates based on individual survey days (i.e., not two-day average) ranged from 0.09 mg/person/day (mean per-capita) to 0.50 mg/person/day at the 95th percentile per-capita intake. On a per-user basis, intake estimates ranged from a mean of 0.34 mg/person/day to 0.81 mg/person/day at the 95th percentile of intake.

II. INTAKE ESTIMATES

A. Food Consumption Data

Detailed information on food and beverages consumed by the U.S. population is collected by the USDA in their Continuing Surveys of Food Intakes by Individuals (CSFII). The most recent survey, conducted between 1994 and 1996 (94-96 CSFII), has been used to estimate intake of lysozyme from selected foods (USDA 1998).

The 94-96 CSFII was conducted as three separate 1-year surveys. Each survey used a stratified area probability sample of individuals residing in all 50 states. The USDA developed statistical weights to adjust for over- and under-representation of certain population subgroups in the unweighted sample due to the sample design, nonresponse, and unequal interviewing across seasons and days of the week. Statistical weights were also developed to allow results of the three years of surveys to be combined for analysis.
Information on the amounts and kinds of foods and beverages consumed at home as well as away from home was collected by an in-person interviewer using a multiple-pass 24-hour recall. Quantities of foods and beverages consumed were recorded in household measures; USDA converted the quantities to grams. Each food reported in the survey was assigned a code by USDA and entered into the survey database; about 6,000 food codes were reported in the survey database.

Approximately 16,000 individuals participated in the surveys over the 3-year period. Individuals who took part in the survey were asked to provide two nonconsecutive days of dietary data. Although most participants reported consumption for both days of the survey, some individuals reported consumption for only one day. Separate statistical weights were developed for consumption data collected on Day 1 of the survey and for data reported by individuals participating in both days of the survey. Intake estimates presented in this report are based on data from only those respondents who provided consumption information for both days of the survey.

B. Proposed Uses and Use Levels

A list of foods included in the data analysis is presented in the Appendix. Estimated intake of lysozyme was based on the proposed use levels of 2.5 mg/lb frankfurter and 2.0 mg/lb cooked meat. Cooked “deli-type” meats such as turkey loaf, roast beef, ham, pastrami and other meats sold ready-to-eat were included in the category cooked meat. Note that, as a conservative estimate, all types of frankfurters (including turkey and chicken) reported consumed in the CSFII were assumed to contain lysozyme. Although the use of lysozyme for frankfurters is limited to the hot dog casing, it was assumed that all lysozyme would be transferred to the hot dog and that no lysozyme would be lost during cooking.

III. RESULTS

Estimated intake of lysozyme by the overall U.S. population is presented in Table 1. Mean, 90th percentile and 95th percentile of intake are reported.
IV. REFERENCE

TABLE 1

INTAKE ESTIMATES: LYSOZYME IN FRANKFURTER CASINGS
AND IN COOKED MEATS
(MG/PERSON/DAY)

<table>
<thead>
<tr>
<th></th>
<th>2-DAY AVERAGE INTAKE</th>
<th>PERSON-DAY INTAKE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PER CAPITA</td>
<td>PER USER</td>
</tr>
<tr>
<td>Mean</td>
<td>0.09</td>
<td>0.21</td>
</tr>
<tr>
<td>90(^{th}) percentile</td>
<td>0.27</td>
<td>0.40</td>
</tr>
<tr>
<td>95(^{th}) percentile</td>
<td>0.38</td>
<td>0.52</td>
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### APPENDIX

**PROPOSED FOOD USES INCLUDED IN THE ESTIMATED INTAKE OF LYSOZYME**

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>22431000</td>
<td>Pork roll, cured, fried</td>
</tr>
<tr>
<td>24198660</td>
<td>Chicken, chicken roll, roasted, NS as to light or dark meat</td>
</tr>
<tr>
<td>24198640</td>
<td>Chicken, chicken roll, roasted, light meat</td>
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<tr>
<td>24198650</td>
<td>Chicken, chicken roll, roasted, dark meat</td>
</tr>
<tr>
<td>24204000</td>
<td>Turkey, roll, roasted</td>
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<tr>
<td>25210110</td>
<td>Frankfurter, wiener, or hot dog, NFS</td>
</tr>
<tr>
<td>25210120</td>
<td>Frankfurter or hot dog, breaded, baked</td>
</tr>
<tr>
<td>25210150</td>
<td>Frankfurter or hot dog, cheese-filled</td>
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<tr>
<td>25210160</td>
<td>Frankfurter or hot dog, bacon and cheese-filled</td>
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<tr>
<td>25210170</td>
<td>Frankfurter or hot dog, chili-filled</td>
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<tr>
<td>25210210</td>
<td>Frankfurter or hot dog, beef</td>
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<tr>
<td>25210220</td>
<td>Frankfurter or hot dog, beef and pork</td>
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<tr>
<td>25210230</td>
<td>Frankfurter or hot dog, beef and pork, lowfat</td>
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<tr>
<td>25210250</td>
<td>Frankfurter or hot dog, meat and poultry, fat free</td>
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<td>25210280</td>
<td>Frankfurter or hot dog, meat and poultry</td>
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<td>25210310</td>
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<td>25210410</td>
<td>Frankfurter or hot dog, turkey</td>
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<td>25210510</td>
<td>Frankfurter or hot dog, low salt</td>
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<td>25210610</td>
<td>Frankfurter or hot dog, beef, lowfat</td>
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<td>25210700</td>
<td>Frankfurter or hot dog, meat &amp; poultry, lowfat</td>
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<td>25220010</td>
<td>Cold cuts, NFS</td>
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<td>25220390</td>
<td>Bologna, beef, lowfat</td>
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<tr>
<td>25220400</td>
<td>Bologna, pork and beef</td>
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<tr>
<td>25220410</td>
<td>Bologna, NFS</td>
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<tr>
<td>25220420</td>
<td>Bologna, Lebanon</td>
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<tr>
<td>25220430</td>
<td>Bologna, beef</td>
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<tr>
<td>25220440</td>
<td>Bologna, turkey</td>
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<td>Bologna ring, smoked</td>
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<td>25220460</td>
<td>Bologna, pork</td>
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<td>25220470</td>
<td>Bologna, beef, lower sodium</td>
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<tr>
<td>25220480</td>
<td>Bologna, chicken, beef, and pork</td>
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<td>25220490</td>
<td>Bologna, with cheese</td>
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<tr>
<td>25220500</td>
<td>Bologna, beef and pork, lowfat</td>
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<tr>
<td>25220510</td>
<td>Capicola</td>
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<td>25221500</td>
<td>Salami, NFS</td>
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<td>Salami, soft, cooked</td>
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<td>Salami, beef</td>
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<td>25230110</td>
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<td>25230210</td>
<td>Ham, sliced, prepackaged or deli, luncheon meat</td>
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<td>Ham, sliced, extra lean, prepackaged or deli, luncheon meat</td>
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<td>25230310</td>
<td>Chicken or turkey loaf, prepackaged or deli, luncheon meat</td>
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<tr>
<td>25230410</td>
<td>Ham loaf, luncheon meat</td>
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<tr>
<td>25230430</td>
<td>Ham and cheese loaf</td>
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<tr>
<td>25230450</td>
<td>Honey loaf</td>
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<tr>
<td>25230510</td>
<td>Ham, luncheon meat, chopped, minced, pressed, spiced, not canned</td>
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<tr>
<td>25230520</td>
<td>Ham, luncheon meat, chopped, minced, pressed, spiced, lowfat, not canned</td>
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<tr>
<td>25230550</td>
<td>Ham, pork, and chicken, luncheon meat, chopped, minced, pressed, spiced, canned, reduced sodium</td>
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<td>25230560</td>
<td>Liverwurst</td>
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<td>25230610</td>
<td>Luncheon loaf (olive, pickle, or pimiento)</td>
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<td>25230710</td>
<td>Sandwich loaf, luncheon meat</td>
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<tr>
<td>25230790</td>
<td>Turkey ham, sliced, extra lean, prepackaged or deli, luncheon meat</td>
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</table>

Prepared for Rhodia, Inc. by Novigen Sciences, Inc
<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
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<tbody>
<tr>
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<td>Veal loaf</td>
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<td>52230840</td>
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<td>52230900</td>
<td>Turkey or chicken breast, prepackaged or deli, luncheon meat</td>
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<tr>
<td>25231110</td>
<td>Beef, sliced, prepackaged or deli, luncheon meat</td>
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<tr>
<td>25231150</td>
<td>Corned beef, pressed</td>
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<tr>
<td>27113200</td>
<td>Creamed chipped or dried beef</td>
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<td>27120210</td>
<td>Frankfurter or hot dog, with chili, no bun</td>
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<td>27120250</td>
<td>Frankfurters or hot dogs with tomato-based sauce (mixture)</td>
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<tr>
<td>27220080</td>
<td>Ham croquette</td>
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<td>27420040</td>
<td>Frankfurters or hot dogs and sauerkraut (mixture)</td>
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<td>27460490</td>
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<td>Antipasto with ham, fish, cheese, vegetables</td>
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<td>27500100</td>
<td>Meat sandwich, NFS</td>
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<td>27510910</td>
<td>Corned beef sandwich</td>
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<td>27510950</td>
<td>Reuben sandwich (corned beef sandwich with sauerkraut and cheese), with spread</td>
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<tr>
<td>27511010</td>
<td>Pastrami sandwich</td>
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<td>Roast beef sandwich</td>
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<tr>
<td>27513020</td>
<td>Roast beef sandwich, with gravy</td>
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<tr>
<td>27513040</td>
<td>Roast beef submanne sandwich, on roll, with lettuce, tomato and spread</td>
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<td>Roast beef sandwich with cheese</td>
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<td>27513060</td>
<td>Roast beef sandwich with bacon and cheese sauce</td>
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<td>Gyro sandwich (pita bread, beef, lamb, onion, condiments), with tomato and spread</td>
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<td>27520250</td>
<td>Ham on biscuit</td>
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<td>27520300</td>
<td>Ham sandwich, with spread</td>
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<td>Ham sandwich with lettuce and spread</td>
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<tr>
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<td>Ham and cheese sandwich, with lettuce and spread</td>
</tr>
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<td>27520330</td>
<td>Ham and egg sandwich</td>
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<tr>
<td>27520340</td>
<td>Ham salad sandwich</td>
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<td>27520350</td>
<td>Ham and cheese sandwich, with spread, grilled</td>
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<td>27520360</td>
<td>Ham and cheese sandwich, on bun, with lettuce and spread</td>
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<tr>
<td>27520370</td>
<td>Hot ham and cheese sandwich, on bun</td>
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<td>27520380</td>
<td>Ham and cheese on English muffin</td>
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<td>27520390</td>
<td>Ham and cheese submanne sandwich, on multigrain roll, with lettuce, tomato and spread</td>
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<td>27520540</td>
<td>Ham and tomato club sandwich, with lettuce and spread</td>
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<td>Turkey sandwich, with spread</td>
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<td>27540330</td>
<td>Turkey sandwich, with gravy</td>
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<td>Turkey submanne sandwich, on roll, with cheese, lettuce, tomato and spread</td>
</tr>
<tr>
<td>27560000</td>
<td>Luncheon meat sandwich, NFS, with spread</td>
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<td>Bologna sandwich, with spread</td>
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<td>27560120</td>
<td>Bologna and cheese sandwich, with spread</td>
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<td>27560390</td>
<td>Corn dog (frankfurter or hot dog with cornbread coating)</td>
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<td>27560310</td>
<td>Corny dog, with chili, on bun</td>
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<tr>
<td>27560320</td>
<td>Frankfurter or hot dog, plain, on bun</td>
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<tr>
<td>27560330</td>
<td>Frankfurter or hot dog, with cheese, plain, on bun</td>
</tr>
<tr>
<td>27560340</td>
<td>Frankfurter or hot dog, with catsup and/or mustard, on bun</td>
</tr>
<tr>
<td>27560350</td>
<td>Pig in a blanket (frankfurter or hot dog wrapped in dough)</td>
</tr>
<tr>
<td>27560360</td>
<td>Frankfurter or hot dog, with chili, on bun</td>
</tr>
<tr>
<td>27560370</td>
<td>Frankfurter or hot dog with chili and cheese, on bun</td>
</tr>
<tr>
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<td>Description</td>
</tr>
<tr>
<td>-------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
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<td>27560380</td>
<td>Pochito (frankfurter or hot dog and beef chili wrapped in tortilla)</td>
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<tr>
<td>27560400</td>
<td>Chicken frankfurter or hot dog, plain, on bun</td>
</tr>
<tr>
<td>27560510</td>
<td>Salami sandwich, with spread</td>
</tr>
<tr>
<td>27560910</td>
<td>Submarine, cold cut sandwich, on bun, with lettuce</td>
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<td>32105030</td>
<td>Egg omelet or scrambled egg, with ham or bacon</td>
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<tr>
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<td>Egg omelet or scrambled egg, with peppers, onion, and ham</td>
</tr>
<tr>
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<td>Egg omelet or scrambled egg, with cheese and ham or bacon</td>
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<tr>
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<td>Egg omelet or scrambled egg, with cheese, ham or bacon, and tomatoes</td>
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</tr>
<tr>
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</tr>
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<td>Egg and ham on biscuit</td>
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<td>Croissant, filled with ham and cheese</td>
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<td>Croissant with ham, egg, and cheese</td>
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<td>Spaghetti in tomato sauce w/frankfurters</td>
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<td>Pasta in tomato sauce w/frankfurters, canned</td>
</tr>
<tr>
<td>58145160</td>
<td>Macaroni or noodles with cheese and frankfurters or hot dogs</td>
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</table>
FR FDA 03/13/98 PR 63 FR 12421 - DIRECT FOOD SUBSTANCES AFFIRMED AS GENERALLY RECOGNIZED AS SAFE; EGG WHITE LYSOZYME

Friday, March 13, 1998 Vol. 63, No. 49 p 12421 (Proposed Rule)

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

21 CFR Part 184

[Docket No. 89 G-0393]

Direct Food Substances Affirmed as Generally Recognized as Safe; Egg White Lysozyme

AGENCY: Food and Drug Administration, HHS.

ACTION: Tentative final rule.

SUMMARY: The Food and Drug Administration (FDA) is issuing a tentative final rule to amend its regulations to affirm that egg white lysozyme enzyme preparation, when labeled by the common or usual name "egg white lysozyme" to identify its source, is generally recognized as safe (GRAS) for use in preventing late blowing of cheese caused by the bacterium Clostridium tyrobutyricum during cheese production. This action is in response to a petition submitted by Fordras S.A. (formerly SPA-Societa Prodotti Antibiotici S.p.A.). FDA has tentatively concluded that this use of the egg white lysozyme enzyme preparation is GRAS only when the ingredient statement for both bulk and packaged food that contains cheese manufactured using egg white lysozyme includes the common or usual name "egg white lysozyme" to identify the source of the protein. To give interested persons an opportunity to comment on this condition of use required for GRAS status, FDA is issuing this tentative final rule.

DATES: Submit written comments by May 27, 1998.

ADDRESSES: Submit written comments to the Dockets Management Branch (HFA-305), Food and Drug Administration, 12420 Parklawn Dr., rm. 1-23, Rockville, MD 20857.

FOR FURTHER INFORMATION CONTACT: Linda S. Kahl, Center for Food Safety and Applied Nutrition (HFS-206), Food and Drug Administration, 200 C St. SW., Washington, DC 20204.
SUPPLEMENTARY INFORMATION:

I. Background

In accordance with the procedures described in Sec. 170.35 (21 CFR 170.35), SPA-Societa Prodotti Antibiotici S.p.A., now Fordras S.A., Milan, Italy, submitted a petition (GRASP 9G0355) requesting that egg white lysozyme used to inhibit the bacterium C. tyrobutyricum to prevent late blowing of cheese during production be affirmed as GRAS as a direct human food ingredient. FDA published the notice of filing for this petition in the Federal Register of October 27, 1989 (54 FR 43861), and gave interested persons until December 26, 1989, to submit written comments.

II. Standards for GRAS Affirmation

Under Sec. 170.30 (21 CFR 170.30), general recognition of safety may be based only on the views of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. The basis of such views may be either: (1) Scientific procedures, or (2) in the case of a substance used in food prior to January 1, 1958, through experience based on common use in food. General recognition of safety based upon scientific procedures requires the same quantity and quality of scientific evidence as is required to obtain approval of a food additive regulation and ordinarily is based upon published studies, which may be corroborated by unpublished studies and other data and information (Sec. 170.30(b)). General recognition of safety through experience based on common use in food prior to January 1, 1958, may be determined without the quantity or quality of scientific procedures required for approval of a food additive regulation, but ordinarily is based upon generally available data and information concerning the pre-1958 history of use of the substance.

FDA has evaluated Fordras S.A.'s petition on the basis of scientific procedures to whether the petitioned use of egg white lysozyme enzyme preparation to prevent the late blowing of cheese caused by the bacterium C. tyrobutyricum during cheese production is GRAS. In evaluating the petition, FDA considered published and unpublished data and information relating to the identity of, characteristic properties of, and estimated dietary exposure to the enzyme component (i.e., lysozyme) of the petitioned enzyme preparation (Refs. 1 through 7). FDA also considered that the source of the petitioned enzyme preparation, egg white, has been safely consumed by humans as a source of food protein throughout recorded history, and, therefore, is GRAS (Sec. 170.30(d)), and that the methods used for extracting lysozyme from the egg white source do not ordinarily alter the chemical identity and characteristic properties of enzymes (Ref. 8). FDA also considered published scientific review articles (Refs. 1 and 2) and a generally available trade association bulletin (Ref. 7) discussing the use of egg white lysozyme enzyme preparation for its technical effect of preventing late blowing of cheese contaminated with C. tyrobutyricum as well as generally available information documenting that this intended use of the petitioned enzyme preparation has been approved in several European countries (Refs. 9 through 13). Finally, FDA considered generally available and accepted information relating to processing aids used in the manufacture of the enzyme preparation and generally available and accepted specifications for food-grade enzyme preparations (Ref. 14).
III. Safety Evaluation

When present as a contaminant in milk used for cheesemaking, the pasteurization-resistant bacterium C. tyrobutyricum ferments lactate to produce carbon dioxide, hydrogen, and volatile organic acids. This fermentation causes a defect in cheese manufacture known as "late blowing," which is typified by abnormal levels of open texture accompanied by undesirable odors and flavors. Late blowing can be a serious economic problem in the manufacture of several varieties of cheese (Refs. 1, 2, and 7).

The contamination by C. tyrobutyricum of milk used for cheesemaking, although reducible by good husbandry and hygienic milking practices, is unavoidable. Although treatment with certain chemical agents has been shown to be effective against the problems raised by this contamination, treatment with lysozyme enzyme preparation has been found to be the most effective method of managing the late blowing of cheese contaminated with C. tyrobutyricum (Refs. 1 and 2).

A. The Enzyme Component

Enzymes are proteins or conjugated proteins (i.e., a protein that contains a nonamino acid moiety such as a carbohydrate) produced by plants, animals, and microorganisms that function as biochemical catalysts (American Heritage Dictionary of the English Language). Most enzymes are very specific in their ability to catalyze only certain chemical reactions; this high degree of specificity and strong catalytic activity are the most important functional properties of enzymes (Ref. 15).

The Commission on Enzymes of the International Union of Biochemistry has devised a systematic strategy for naming enzymes. This system combines a naming system and a numbering system. For most enzymes, the systematic name is derived from the names of the substrate, product, and type of reaction. The systematic name is based on the class and subclasses to which the enzyme belongs. The systematic name of lysozyme is peptidoglycan N-acetylmuramoylhydrolase. Its systematic number is EC No. 3.2.1.17 and its Chemical Abstracts Service Registry Number (CAS Reg. No.) is 9001-63-2.

Lysozyme was first discovered by A. Fleming, who identified lysozyme as an antibacterial enzyme present in nasal mucus membrane (Ref. 3). Subsequently, it was learned that the antibacterial activity of lysozyme occurs because of its ability to catalyze the hydrolysis of the structural polysaccharide peptidoglycan present in cell walls of certain bacteria (Ref. 2). Lysozyme activity has been shown to be present in bacteria, fungi, plants, and almost all animal tissues, with the highest levels found in secretions (including milk, mucus, saliva, and tears) and eggs. Lysozyme is believed to function in all of these organisms and tissues as an endogenous antimicrobial substance (Refs. 1 and 2).

Lysozyme was the first enzyme to have the details of its three-dimensional structure published (Ref. 4), and it has become one of the best characterized of all enzymes, serving as an example for studies of enzyme mechanism and molecular evolution (Refs. 5 and 6). Lysozymes from various organisms are very similar to one another. Egg white lysozyme differs very little in structure, amino acid sequence and composition, catalytic mechanism, and substrate specificity from the enzyme found in human milk, saliva, mucus, and tears (Refs. 3 and 6).

The petitioner provided two published scientific review articles (Refs. 1 and 2) that discuss the use of egg white lysozyme in cheese and other food. The petitioner also provided a generally available trade
association bulletin (Ref. 7) that focuses on the use of egg white lysozyme for its technical effect of preventing late blowing in cheese. This bulletin describes the late blowing defect and how it arises, traditional chemical control measures (other than the use of lysozyme) to reduce the problem, and the increasing interest in using lysozyme as a replacement for traditional chemical control measures. In addition, the petitioner provided generally available information documenting that this intended use of the petitioned enzyme preparation has been approved in several countries, including Denmark, France, Germany, Italy, and Spain (Refs. 9 through 13).

FDA considered the estimated dietary exposure to lysozyme for the proposed use in cheese (Refs. 16 and 17). Lysozyme accounts for approximately 3.5 percent of the total protein of domestic hen egg whites (Ref. 7). Whole eggs contain lysozyme at a level of approximately 3,300 parts per million (ppm). The petitioner reported that cheese manufactured using egg white lysozyme enzyme preparation contains a maximum of 400 ppm of lysozyme, or at least 8 times less than eggs on a weight basis. FDA has estimated a long-term mean intake of lysozyme to be 74 milligrams per person per day (mg/p/d) for consumers of eggs and 3.8 mg/p/d for consumers of cheese; the respective 90th percentile intakes are estimated to be 163 mg/p/day and 8.1 mg/p/day. Egg whites from which lysozyme is extracted will be subsequently consumed in other food uses. Thus, there will be no long-term net increase in lysozyme intake by the general population because egg whites without lysozyme will replace egg whites in current use that contain lysozyme (Ref. 16). On a per eating occasion basis, lysozyme intake for cheese consumers may be 16 mg on average, or 22 mg at the 90th percentile level. For comparison, a per eating occasion lysozyme intake for egg consumers may be 264 mg on average, or 416 mg at the 90th percentile level. Thus, lysozyme intake per eating occasion due to cheese consumption may constitute 5 to 6 percent of lysozyme intake due to egg consumption (Ref. 17).

In general, issues relevant to a safety evaluation of proteins such as the enzyme component of an enzyme preparation are potential toxicity and allergenicity (Ref. 18). Proteins derived from egg whites do not raise toxicity concerns because egg whites have been safely consumed by humans as a source of food throughout recorded history without any reports of toxicity. However, proteins derived from egg whites do raise allergenicity concerns because, as with many common foods, there have been reports that consumption of egg whites can cause an allergic reaction in certain individuals, particularly children (Ref. 19). Therefore, FDA considered the question of whether the lysozyme component of egg whites is allergic.

In evaluating this question, FDA considered a report of an in vitro study of the binding of antibodies to specific egg proteins, where the antibodies were derived from the serum of patients known to be allergic to eggs (Ref. 20). This report suggests that lysozyme was an allergen for some individuals who became sensitive to egg whites. Although this study does not establish that ingestion of egg white lysozyme in cheese will actually cause a clinically significant allergic reaction in such sensitive individuals, FDA is not aware of any data or information that would refute the study's inference that egg white lysozyme may be allergenic. Accordingly, FDA is proposing labeling, as discussed below, to alert the sensitive population to the presence of egg white lysozyme in cheese:

A related question is whether egg white lysozyme, when present in cheese, is capable of inducing an
allergic response in susceptible individuals who have not previously consumed egg whites, e.g., because their customary diet excludes eggs. This question is no different than for any other food containing egg white when consumed by individuals with unknown susceptibility to eggs. The proposed label declaration would provide such individuals with the same protection as that provided by other egg-containing products with ingredient labeling. Thus, individuals who experience an allergic reaction to lysozyme-containing cheese could identify egg white lysozyme as a possible cause of the reaction.

B. Enzyme Source, Manufacturing Methods, and Processing Aids

Commercial preparations of lysozyme are derived from domestic hen egg whites using ion exchange methods and selective precipitation to isolate a highly purified protein fraction that contains mainly lysozyme but also may contain small amounts of other egg white proteins. Consistent with the agency's finding in its GRAS affirmation of microparticulated protein product (55 FR 6384, February 23, 1990), FDA finds that egg whites have been safely consumed by humans throughout recorded history and, therefore, are GRAS (Sec. 170.30(d)). The agency evaluated the methods used to isolate the enzyme lysozyme from egg whites. These methods are based on generally available and accepted principles of protein purification (Ref. 8). Such methods, if appropriately selected, do not ordinarily alter the chemical identity and characteristic properties of enzymes. Therefore, these methods do not materially change the quality, utility, functionality, or safety of enzymes. Moreover, the retention of the antibacterial activity that is characteristic of egg white lysozyme when egg white-derived lysozyme enzyme preparation is used in cheese evidences that lysozyme in the manufactured enzyme preparation remains unaltered from the lysozyme in egg whites. This is corroborative evidence of the fact that the methods used to isolate lysozyme from egg whites do not materially change the quality, utility, functionality or safety of the enzyme lysozyme. Enzyme preparations used in food processing are usually not chemically pure but contain, in addition to the enzyme component, materials that derive from the enzyme source. As mentioned above, egg white lysozyme enzyme preparation may contain small amounts of other egg white proteins. A related question is whether such proteins that may be present in the enzyme preparation are allergenic. Even if present, other source-derived proteins would not be a concern because the proposed label declaration for egg white lysozyme would alert individuals who are sensitive to egg whites to the possible presence of other proteins derived from egg whites.

In addition to source-derived materials, enzyme preparations used in food processing usually contain materials that derive from the manufacturing methods used to generate the finished enzyme preparation. The egg white lysozyme enzyme preparation that is the subject of this document complies with the general requirements and additional requirements for enzyme preparations in the Food Chemicals Codex, 4th ed. (Ref. 14). The egg white lysozyme enzyme preparation that is the subject of this document may contain substances that are added to the enzyme preparation, such as preservatives, stabilizers or diluents, and trace amounts of processing aids that are used in its preparation. These substances must be acceptable for general use in foods (Refs. 14 and 15).

C. Labeling as a Condition of Use

Egg whites are known to be an allergenic food source, particularly in children (Ref. 19). There is a literature report (Ref. 20) indicating that lysozyme may in fact have been an allergen for some individuals who became sensitive to egg whites. Although the reported in vitro study does not establish that ingestion of egg white lysozyme in cheese will actually cause a clinically significant
allergic reaction in such sensitive individuals, FDA is not aware of any data or information that would refute the study's inference that egg white lysozyme may be allergenic. Therefore, FDA concludes that there is insufficient information in the current record to determine whether the ingestion of egg white lysozyme elicits an allergic response when consumed by individuals who are sensitive to egg whites. Accordingly, as discussed below, FDA is proposing labeling to alert such individuals to the presence of egg white lysozyme in cheese. Such labeling also would alert the sensitive population to the possible presence of source-derived proteins other than lysozyme in the enzyme preparation.

Under section 409(c)(1) of the Federal Food, Drug, and Cosmetic Act (the act) (21 U.S.C. 348(c)(1)), FDA is authorized, in approving the use of a food additive, to list the conditions under which the additive may be safely used. These conditions may include any labeling requirements that the agency deems necessary to ensure the safe use of the additive. Similarly, under Sec. 184.1(b)(3) (21 CFR 184.1(b)(3)), in affirming a substance as GRAS, FDA is authorized to set forth the particular conditions of use, including labeling, under which there is general recognition among qualified experts that the use of the substance is safe. After careful review of the evidence on the use of egg white lysozyme enzyme preparation in preventing late blowing in cheese, FDA has tentatively concluded that such use is GRAS only when the conditions of its use include a declaration on the label or labeling of the presence of egg white lysozyme in both bulk and packaged food containing such treated cheese. Therefore, this tentative final rule (Sec. 184.1550(c)(1)) establishes that the declaration of egg white lysozyme enzyme preparation by the common or usual name "egg white lysozyme" is a condition of use required for GRAS status, so that consumers who are allergic to egg white products can be alerted to the presence of the egg white-derived enzyme in treated cheese.

D. Summary and Conclusions

The petitioner provided published data and information relating to the identity of, characteristic properties of, and estimated dietary exposure to the enzyme component (Refs. 1 through 7). The source of the petitioned enzyme preparation, egg white, has been safely consumed by humans as a source of food protein throughout recorded history, and, therefore, is GRAS (Sec. 170.30(d)). The petitioner provided generally available information showing that the methods used for extracting lysozyme from the egg white source do not ordinarily alter the chemical identity and characteristic properties of enzymes (Ref. 8). Moreover, there is corroborating evidence that the extraction of egg white lysozyme does not change its chemical identity or characteristics because the antibacterial activity of egg white lysozyme is retained. FDA concludes that the methods used to manufacture egg white lysozyme enzyme preparation do not change the safety for food use of the enzyme lysozyme and that toxicological studies are not necessary to establish the safety of lysozyme or other source-derived proteins that may remain in the manufactured enzyme preparation. FDA also concludes that there will be no net increase in dietary exposure of the general population to the commonly consumed enzyme lysozyme due to the proposed use in cheese because lysozyme will simply be transferred from eggs to cheese (Ref. 16).

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The petitioner also provided generally available and accepted information relating to processing aids used in the manufacture of the enzyme preparation and generally available and accepted specifications for food grade enzyme preparations (Ref. 14). FDA concludes that substances added to the egg white
lysozyme enzyme preparation or potential residues of processing aids used in the manufacturing process do not present a basis for concern about the safety of the egg white lysozyme enzyme preparation.

The petitioner provided published scientific review articles (Refs. 1 and 2) and a generally available trade bulletin (Ref. 7) that discuss the use of the egg white lysozyme enzyme preparation in cheese and other food, including its use for the intended effect of preventing late blowing of cheese contaminated with C. tyrobutyricum. The petitioner also provided generally available information documenting that this intended use of lysozyme has been approved in several European countries (Refs. 9 through 13). FDA concludes that generally available and accepted data and information establish that lysozyme will achieve the intended technical effect of preventing late blowing in cheese contaminated with C. tyrobutyricum.

Finally, information in the petition and otherwise available to FDA raises the question of whether the lysozyme component of egg whites is allergenic. FDA is proposing labeling to alert individuals who may be sensitive to egg whites to the presence of egg white lysozyme in cheese, including the possible presence of other source-derived proteins that may be present in the enzyme preparation.

IV. Comments

FDA received two comments in response to the filing notice. One comment expressed agreement that lysozyme is GRAS for use in preventing late blowing in cheese and supported the affirmation of GRAS status by the agency.

One comment stated that use of lysozyme as a food preservative may lead to selection of lysozyme-resistant strains of the bacterial food poisoning agents Listeria monocytogenes and C. botulinum, rendering one of the body’s main defense mechanisms useless against resistant strains. The comment likened the potential selection of lysozyme-resistant strains of bacteria to the selection of penicillin-resistant bacteria as a result of its widespread use. The comment pointed out that the body could not readily substitute the lysozyme naturally present in secretions such as tears and saliva for another antimicrobial.

The mechanism of action of lysozyme involves hydrolysis of the structural peptidoglycan present in cell walls of susceptible bacteria. Therefore, development of resistance to lysozyme would require that a bacterium develop a variant of peptidoglycan that is resistant to the action of lysozyme. Development of such a variant peptidoglycan is, in principle, possible. However, as already discussed, lysozyme activity has been shown to be present in bacteria, fungi, plants, and almost all animal tissues. If such relative ubiquity has not resulted in the clinically significant selection of lysozyme-resistant bacteria to date, the use of lysozyme in those cheeses that are susceptible to late blowing is unlikely to favor selection of lysozyme-resistant bacteria and adversely affect the public health. Moreover, FDA is not considering lysozyme for use as a widespread food preservative. Rather, FDA is considering the narrow question of whether the use of lysozyme in preventing late blowing in cheese is generally recognized as safe. FDA disagrees that this limited use in cheese is analogous to the widespread use of antibiotics such as penicillin and the subsequent selection of antibiotic-resistant bacterial strains. Therefore, FDA concludes that the use of lysozyme in preventing late blowing in cheese does not raise concerns about the selection of lysozyme-resistant strains of L. monocytogenes or C. botulinum.

V. Specifications

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The agency finds that, because the potential impurities in the egg white lysozyme preparation that may originate from the source or manufacturing process do not raise any basis for concern about the safe use of the preparation, the general requirements and additional requirements for enzyme preparations in the monograph on Enzyme Preparations in the Food Chemicals Codex, 4th ed. (1996), which are being incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51, are adequate as minimum criteria for food-grade egg white lysozyme enzyme preparation. Lysozyme assay can be performed using a method entitled "Lysozyme hydrochloride, Microbiological Determination," which is included in the petition (Ref. 21) or by using any appropriate validated method.

VI. Conclusions

The agency has evaluated all available information and finds, based upon the published information about the manufacturing methods used in the preparation of egg white lysozyme enzyme preparation, and published data and information about the identity and characteristic properties of egg white lysozyme, that the enzyme component of egg white lysozyme enzyme preparation is unaltered from the lysozyme found in the commonly consumed food, eggs. The agency also finds, based upon generally available and accepted information, that when the preparation is manufactured in accordance with Sec. 184.1550(c), the source, egg whites, and the manufacturing process will not introduce impurities into the preparation that may render its use unsafe. Further, the agency finds, based upon published information, that egg white lysozyme enzyme preparation will achieve its intended technical effect of preventing late blowing in cheese contaminated with C. tyrobutyricum. Therefore, the agency tentatively concludes, based upon the evaluation of published data and information, corroborated by unpublished data and information, that the egg white lysozyme enzyme preparation described in the regulation set out below is GRAS for use by the general population in preventing late blowing in cheese.

To give interested persons an opportunity to comment on the proposed label declaration that is a condition of use required for GRAS status, FDA is issuing this tentative final rule under 21 CFR 10.40(f)(6). FDA will review any comments that are relevant to this condition of use and that are received within the 75 day comment period and will respond accordingly to these comments in the Federal Register.

VII. Environmental Considerations

The agency has carefully considered the potential environmental effects of this action. FDA has concluded that the action will not have a significant impact on the human environment, and that an environmental impact statement is not required. The agency's finding of no significant impact and the evidence supporting that finding, contained in an environmental assessment, may be seen in the Dockets Management Branch.

VIII. Analysis of Economic Impacts
A. Benefit-Cost Analysis

FDA has examined the impacts of this tentative final rule under Executive Order 12866. Executive Order 12866 directs Federal agencies to assess the costs and benefits of available regulatory alternatives, and, when regulation is necessary, to select regulatory approaches that maximize net benefits (including potential economic, environmental, public health and safety effects; distributive impacts; and equity). According to Executive Order 12866, a regulatory action is "significant" if it meets any one of a number of specified conditions, including having an annual effect on the economy of $100 million, adversely affecting in a material way a sector of the economy, competition, or jobs, or if it raises novel legal or policy issues. FDA finds that this tentative final rule is not a significant regulatory action, as defined by Executive Order 12866. In addition, it has been determined that this final rule is not a major rule for the purpose of congressional review.

The primary benefit of this action is to remove uncertainty about the regulatory status of the petitioned substance. FDA is tentatively affirming the GRAS status of egg white lysozyme in cheese only when the ingredient statement of the bulk and packaged food that contains the cheese includes the common or usual name of the substance, i.e., "egg white lysozyme." The labeling requirement will add a small cost to the future use of the petitioned substance, and therefore, is not a significant action under the Executive Order 12866.

FDA has examined the impacts of this tentative final rule under the Unfunded Mandates Reform Act of 1995 (UMRA) (Pub. L. 104-4). A written statement under section 202(a) of the UMRA is not required for this rule because the rule does not impose a mandate that results in an expenditure of $100 million or more by State, local, and tribal governments in the aggregate, or by the private sector, in any 1 year.

B. Regulatory Flexibility Act

FDA has evaluated this tentative final rule under the Regulatory Flexibility Act. The Regulatory Flexibility Act (5 U.S.C. 601-612) requires Federal agencies to consider alternatives that would minimize the economic impact of their regulations on small entities.

FDA believes that this tentative final rule is not likely to have a significant economic impact on a substantial number of small entities. However, the agency seeks comment on this tentative conclusion. First, FDA is tentatively affirming the GRAS status of egg white lysozyme in cheese only when the ingredient statement of the bulk and packaged food that contains the cheese includes the common or usual name of the substance, i.e., "egg white lysozyme." This labeling requirement will impose only minimal costs to the future use of the petitioned substance. Second, FDA has information that the petitioner does not currently sell egg white lysozyme in the United States (Refs. 22 and 23). Moreover, FDA is not aware of any manufacture or use of cheese containing egg white lysozyme in the United States. If no small entities are currently manufacturing or using cheese containing egg white lysozyme, the proposed labeling requirements would not impose any cost to small entities. However, because FDA does not have any information on whether other entities in the United States are manufacturing or using cheese containing egg white lysozyme, FDA is unable to conclude, in this tentative final rule, that there will be no significant economic impact on a substantial number of small entities. Therefore, the agency seeks comment on the manufacture or use, by any small entity, of cheese containing egg white lysozyme. In its final rule, the agency will, based on any relevant comments received, determine whether there is a significant economic impact on a substantial number of small entities.
of small entities.

IX. References

The following references have been placed on display in the Dockets Management Branch (address above) and may be seen by interested persons between 9 a.m. and 4 p.m., Monday through Friday.


16. Memorandum dated March 20, 1990, from Food and Color Additives Review Section, FDA, to Direct Additives Branch, FDA, "Use of Lysozyme to Prevent the 'Late Blowing' of Cheese."

17. Memorandum dated August 5, 1996, from Chemistry Review Branch, FDA, to Biotechnology Policy Branch, FDA.


21. Lysozyme Hydrochloride, Microbiological Determination.


List of Subjects in 21 CFR Part 184

Food ingredients, Incorporation by reference.

Therefore, under the Federal Food, Drug, and Cosmetic Act and under

[Page 12426]

authority delegated to the Commissioner of Food and Drugs and redelegated to the Director, Center for Food Safety and Applied Nutrition, it is proposed that 21 CFR part 184 be amended as follows:

PART 184--DIRECT FOOD SUBSTANCES AFFIRMED AS GENERALLY RECOGNIZED AS SAFE

1. The authority citation for 21 CFR part 184 continues to read as follows:


2. Section 184.1550 is added to subpart B to read as follows:

Sec. 184.1550 Egg white lysozyme.
(a) Egg white lysozyme (CAS Reg. No. 9001-63-2) is the enzyme peptidoglycan N-acetylmuramoylhydrolase (EC No. 3.2.1.17) obtained by extraction from egg whites. The enzyme catalyzes the hydrolysis of peptidoglycan in the cell walls of certain bacteria including Clostridium tyrobutyricum.

(b) The ingredient meets the general requirements and additional requirements for enzyme preparations in the monograph on Enzyme Preparations in the Food Chemicals Codex, 4th ed. (1996), which is incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies are available from the National Academy Press, 2101 Constitution Ave. NW., Washington, DC 20418, and may be examined at the Center for Food Safety and Applied Nutrition's library, 200 C St. SW., rm. 3321, Washington DC, or at the Office of the Federal Register, 800 North Capitol St. NW., suite 700, Washington, DC.

(c)(1) The ingredient is used in cheeses, as defined in Sec. 170.3(n)(5) of this chapter, in accordance with Sec. 184.1(b)(3) at levels not to exceed current good manufacturing practice.

(2) The affirmation of the use of this ingredient as generally recognized as safe (GRAS) as a direct human food ingredient is based upon the following conditions of use:

(i) The ingredient is used as an enzyme as defined in Sec. 170.3(o)(9) of this chapter.

(ii) Current good manufacturing practice utilizes a level of the ingredient sufficient to prevent the late blowing of cheeses caused by the bacterium Clostridium tyrobutyricum during cheese production.

(iii) The ingredient statement for both bulk and packaged food that contains cheese manufactured using egg white lysozyme shall include the common or usual name "egg white lysozyme" to identify the source of the protein.

L. Robert Lake,
Director, Office of Policy, Planning and Strategic Initiatives, Center for Food Safety and Applied Nutrition.
[FR Doc. 98-6571 Filed 3-12-98; 8:45 am]
BILLING CODE 4160-01-F
BLANK PAGE INSERTED FOR CORRECT PAGINATION
February 9, 2001

Dr. Andrew Laumbach  
Office of Premarket Approval (HFS-200)  
Center for Food Safety and Applied Nutrition  
Food and Drug Administration  
200 C St., SW  
Washington, DC 20204

Re: Egg White Lysozyme GRAS Notice (GRN) No. 000064

Dear Dr. Laumbach:

This will follow up the recent conference call between Dr. Kahl, your other FDA colleagues and yourself, and Jim Elfstrum of my client, Rhodia Inc., and myself regarding the above referenced GRN No. 000064 filed on behalf of Rhodia, Inc. relating to specified uses of egg white lysozyme. Specifically, this letter is intended to address and clarify matters you and your colleagues raised during the call regarding GRN No. 000064.

You noted that the specifications listed on page 000008 do not conform in several respects with the specifications in FDA’s tentative final rule for egg white lysozyme, published on March 13, 1998 (63 FR 12421 et.seq.), including the general requirements and additional requirements for enzyme preparations of the Food Chemicals Codex, 4th ed. We acknowledge these inadvertent inconsistencies in the notice. Consequently, please be advised that the lead limit of the specification on page 000008 should have been not more than 5 ppm. Also, missing from the specification list on page 000008 was a coliform limit of not more than 30 per gram, and a Salmonella sp. limit of negative by test. Therefore, we ask that you utilize the aforementioned corrected limits in your review of GRN No. 000064 and disregard any limits on page 000008 that are inconsistent therewith.

Finally, you noted that a commercial product specification sheet on page 000030 is missing limits for lead, coliform, and Salmonella sp. Again, these limits were inadvertently omitted in the preparation of the specification sheet. Consequently, attached please find a corrected and complete commercial specification sheet containing the aforementioned omitted limits that are consistent both with the FDA tentative final rule for egg white lysozyme, and the general and additional requirements for enzyme preparations of the Food Chemicals Codex, 4th ed. Therefore, we ask that you disregard the specifications contained on page 000030 of GRN No. 000064 and, instead, refer to the enclosed corrected commercial specification sheet in your review of the notice.
The above should fully respond and clarify the matters you raised. We apologize for the inadvertent inconsistencies and omissions contained in the original submission of GRN No. 000064, but appreciate your promptly bringing them to our attention and for this opportunity to correct and clarify them for your review. Please promptly contact me should you have other questions. Thank you.

Sincerely,

Robert H. Sindt

RHS/bs

Enc.
PRODUCT SPECIFICATION – ANTIMICROBIAL PRODUCTS

PRODUCT: Novagard (Lysozyme Chloride)

DESCRIPTION: Food Grade Lysozyme Chloride derived from chicken egg white. (Muramidase, Mucoproteolysis N-acetylmuramoylhydrolase; E.C. No. 3.2.1.17)

STORAGE: In a tightly closed container at cool temperature.

<table>
<thead>
<tr>
<th>SPECIFICATION</th>
<th>LIMIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance:</td>
<td>Dialyzed, white to off-white powder</td>
</tr>
<tr>
<td>Activity *</td>
<td>Minimum 95% (min. 20,000 Shugar units/mg)</td>
</tr>
<tr>
<td>Ash:</td>
<td>Maximum 2.0%</td>
</tr>
<tr>
<td>Moisture</td>
<td>Maximum 5.0%</td>
</tr>
<tr>
<td>pH:</td>
<td>3.5 - 4.5</td>
</tr>
<tr>
<td>Solubility:</td>
<td>Minimum 95% T (1.5% solution OD @ 640 nm)</td>
</tr>
<tr>
<td>Bulk Density:</td>
<td>Minimum 0.5 g/ml</td>
</tr>
<tr>
<td>Chloride:</td>
<td>Maximum 3.5%</td>
</tr>
<tr>
<td>Heavy Metals:</td>
<td>NMT 10 ppm</td>
</tr>
<tr>
<td>Arsenic:</td>
<td>NMT 2 ppm</td>
</tr>
<tr>
<td>Lead:</td>
<td>NMT 5 ppm</td>
</tr>
<tr>
<td>Total Plate Count:</td>
<td>NMT 100/g (FDA/BAM 7, 3)</td>
</tr>
<tr>
<td>Yeast &amp; Mold:</td>
<td>NMT 10/g (FDA/BAM 7, 18)</td>
</tr>
<tr>
<td>Coliforms:</td>
<td>NMT 30/g (FDA/BAM 7, 4)</td>
</tr>
<tr>
<td>Salmonella sp.:</td>
<td>Negative by Test (FDA/BAM 7, 5)</td>
</tr>
</tbody>
</table>

* Activity determined by two procedures:

1. Units/mg Solid = \( \frac{A_{450} \text{ Minute}}{0.001 \times \text{mg solid/Reaction Mixture}} \)

Unit Definition: One Unit is the amount of enzyme which causes a decrease in absorbance of 0.001 per minute at 450 nm at pH 6.2 and 25°C.

2. Compared to Standard of The National Institute of Hygienic Sciences where 1 mg of Standard contains 1 mg lysozyme (potency), after drying.

CONFIDENTIAL
Pages 000089 - 000094 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.
February 21, 2000

Dr. Andrew Laumbach
Office of Premarket Approval (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C St., SW
Washington, DC 20204

Re: Egg White Lysozyme GRAS Notice (GRN) No. 000064

Dear Dr. Laumbach:

This will follow up, clarify, and confirm the matters we discussed in your recent call regarding the above referenced GRN No. 000064 for egg white lysozyme, filed on behalf of my client, Rhodia, Inc.

First, you inquired about Rhodia's corrected commercial specification sheet, submitted to you in my letter of February 9, 2000, which contains a marking at the end of the sheet indicating it to be confidential. Although such a marking is appropriate for the specification sheet's intended commercial uses, Rhodia is aware that such a document, when submitted in support of a GRAS notice, may be subject to public disclosure should FDA receive such a request. Consequently, Rhodia asks that you disregard the document's confidentiality marking for purposes of your review of GRN No. 000064, or for valid requests FDA may receive for public production of the specification sheet.

Second, you inquired about the dossier (Appendix 2) that is referenced in Rhodia's independent expert panel's GRAS report on page 000022, relating to published and unpublished data on the safety of egg white lysozyme. As we discussed, the dossier (Appendix 2) was not included in the GRAS notice submission, but is a part of the file which I have retained and which is available for FDA inspection upon request. Generally, it was not felt necessary to include the full dossier in the GRAS notice, especially since the scientific data relied upon was comprehensively listed in FDA's tentative final rule which was included in the notice on pages 000049 and 000050. This should clarify the matter, although the full file remains available for FDA's inspection upon request.
Please let me know, if you have other questions, so that we may promptly respond to them. Thank you.

Robert H Sindt

RHS:bs
# Reference List for Industry Submission, GRN 000064

<table>
<thead>
<tr>
<th>Pages</th>
<th>Author</th>
<th>Title</th>
<th>Publish Date</th>
<th>Publisher</th>
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<td></td>
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<td>Codex</td>
<td></td>
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</table>

*NA - Not applicable*