ROBERT H. SINDT
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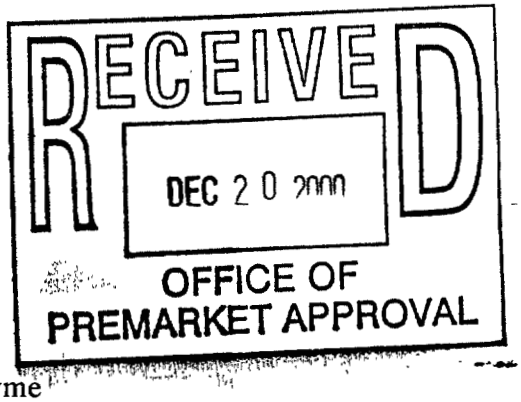
December 19, 2000



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

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Enc.
RHS/bs

ROBERT H. SINDT
ATTORNEY AT LAW

1850 M Street, N.W., Suite 400
Washington, D.C. 20036
Phone 202-466-4500 • Fax 202-466-5777 • E-mail rsindt@krooth.com

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 Nisin

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, all submitted in triplicate, for that purpose as they relate to the use of nisin in certain foods. Specifically, Rhodia claims that use of nisin 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: Nisin, whose Chemical Abstract Service (CAS) registry number is 1414-45-5, and whose specifications conform to those set out at 21 CFR 184.1538.

(iii) Applicable Conditions of Use: Nisin 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 at 21 CFR 184.1538. Nisin is proposed for use as an antimicrobial agent in casings for frankfurters at a concentration of 3.15 mg nisin/lb of frankfurter, approximately 7 mg nisin/kg of food, and for use as an antimicrobial agent on cooked meat and poultry products

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sold ready-to-eat at 2.5 mg nisin/lb of cooked meat or poultry product, approximately 5.5 mg nisin/kg of food. The overall US population is 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 nisin 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\Nisin GRAS Notice Claim.doc

NISIN
GRAS NOTICE INFORMATION

Rhodia

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NISIN-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: Nisin
- Chemical Name for Nisin: None
- Chemical Abstract Service (CAS) Registry Number for Nisin: 1414-45-5.
- Empirical Formula for Nisin: $C_{143}H_{230}N_{42}O_{37}S_7$.
- Structual Formula for Nisin:

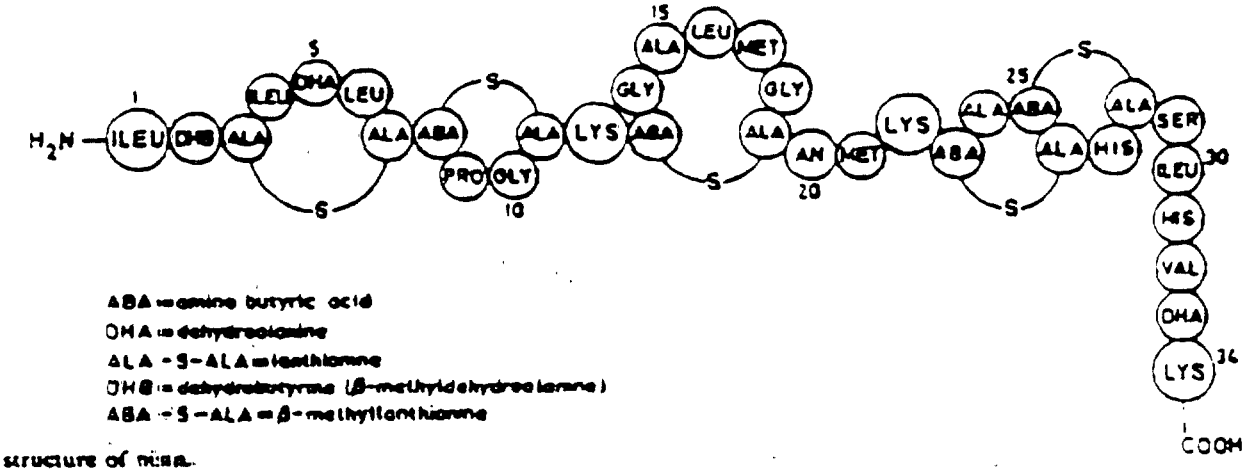

Fig. 1. The structure of nisin.

Journal of the Society of Dairy Technology. Vol. 43, No. 3, August 1990

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- Quantitative Composition for Nisin: Nisin is a commercially available food ingredient as a standardized blend, complying in all respects with the specifications contained in 21 CFR 184.1538 for the substance identified as "nisin preparation" for use in certain cheese products. Nisin is a concentrate or dry material that is standardized with GRAS food substances, such as sodium chloride, to produce an activity level of not less than 1 million IU of nisin per gram as measured by the British Standards Institution Methods, "Methods for the Estimation and Differentiation of Nisin in Processed Cheese, " BS4020 (1974), a copy of which is contained in the Appendix hereto.

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

Specifically, a fermentation medium including yeast extract, corn steep liquor, soy protein peptone and starch, lactose, or dextrose is heat-treated in high grade stainless steel fermentation tanks to achieve sterilization. The medium is cooled to an appropriate incubation temperature and inoculated with a pure culture of nisin-producing *Lactococcus lactis spp lactis*. The fermentation is allowed to progress under controlled time and temperature conditions until optimum nisin production has occurred. At the appropriate time, the fermentation is stopped by chilling and/or heat treatment and then concentrated via alternative processes, which may include foaming precipitation, sodium chloride precipitation, precipitation under acid

conditions, or concentration by ultra-filtration. The resulting nisin concentrate is then centrifuged, and either dried in an oven at 140°F (60°C) for up to four hours, or dried in a spray drier at temperatures of up to 180°F. The dried concentrate is then standardized with sodium chloride and yeast extract or nonfat milk solids to achieve a minimum of 1,000,000 IU nisin activity per gram.

- Characteristic Properties of Nisin: Nisin is a small antimicrobial peptide (MW: 3553 Da) produced by certain strains of *Lactococcus lactis spp lactis*, a common lactic acid producing bacterium. It has been shown to possess antimicrobial properties against select Gram positive bacteria.

- Content of Potential Human Toxicants for Nisin: None.

- Specifications for Food Grade Nisin: Nisin conforms to the specification for "nisin preparation" set forth by the FDA in 21 CFR 184.1538. Nisin meets the specifications that follow when it is tested as described in "Specifications for Identity and Purity of Some Antibiotics," WHO/FAO Nutrition Meeting Report Series, No. 45 A, 1969:

  Nisin content, not less than 900 IU/mg (ie, 900,000 IU/gm)

  Arsenic, not more than 1 ppm

  Lead, not more than 2 ppm

  Zinc, not more than 25 ppm

  Copper; zinc plus copper, not more than 50 ppm

Total plate count, not more than 10/gram

Escherichia coli, absent in 10 grams

Salmonella, absent in 10 grams

Coagulase positive staphylococci, absent in 10 grams.

Further, in compliance with 21 CFR 184.1538, nisin concentrate is standardized with GRAS food substances to produce an activity level of not less than 1 million IU of nisin per gram as measured by the British Standards Institution Methods, "Methods for the Estimation and Differentiation of Nisin in Processed Cheese," BS 4020 (1974).

NISIN-GRAS NOTICE INFORMATION

(3) INFORMATION ON SELF-LIMITING LEVELS OF USE, IF ANY (§170.36(c)(3))

- No information on self limiting levels of nisin use is noted.

NISIN-GRAS NOTICE INFORMATION

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

Rhodia's determination, that the notified uses of nisin (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 Nisin:

Nisin, for the uses proposed herein, meets the specifications for the substance approved by FDA for use in cheese products as specified at 21 CFR 184.1538. Further, it is noted that additional GRAS uses of nisin have been asserted through the filing of GRAS affirmation petitions that have been accepted for filing by FDA: Docket No. 94 G-0027, a GRAS petition for the use of nisin as an antimicrobial agent in reduced cholesterol liquid whole eggs which was accepted by FDA for filing on March 24, 1994 (59 FR 13971); Docket No. 94 G-0272, a GRAS petition for the use of nisin as an antimicrobial agent in various standardized and nonstandardized liquid egg products which was accepted by

FDA for filing on August 17, 1994 (59 FR 42277); and Docket No. 95 G-0389, a GRAS petition for the use of nisin as an antimicrobial agent in sauces and nonstandardized salad dressings which was accepted by FDA for filing on December 14, 1995 (60 FR 64167).

In connection with its determination, Rhodia requested Novigen Sciences, Inc. to conduct an estimated dietary intake assessment for the proposed uses of nisin. 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 nisin by the U.S. population and selected population subgroups in support of a self-assessment of GRAS status. Nisin is proposed for use in casings for frankfurters at concentrations of 3.15 mg nisin/lb frankfurter (approximately 6.9 mg nisin/kg food) and in cooked meats sold ready-to-eat at 2.5 mg nisin/lb cooked meat (approximately 5.5 mg nisin/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 0.58 mg/person/day at the mean to 1.42 mg/person/day at the 95thpercentile of intake. On a per-capita basis, intakes for the U.S. population ranged from 0.24 mg/person/day at the mean to 1.04 mg/person/day at the 95th percentile.

"Intake estimates based on individual survey days (i.e., not two-day average) ranged from 0.24 mg/person/day (mean per-capita) to 1.38

mg/person/day at the 95th percentile per-capita intake. On a per-user basis, intake estimates ranged from a mean of 0.94 mg/person/day to 2.24 mg/person/day at the 95th percentile of intake."

The independent expert panel convened by Rhodia reviewed the Novigen intake assessment report, as well as an FDA memorandum (included in the Appendix hereto) of November 9, 1984 authored by Dr. Alfred N. Milbert regarding the 'Safety and Establishment of ADI for Nisin'. In its report, the independent expert panel noted that "The FDA in 1988 critically evaluated available information on nisin, confirmed the GRAS status of nisin, and reported that nisin is safe for human consumption at an Acceptable Daily Intake (ADI) of 2.94 mg/per/day. The ADI was based on a critical re-evaluation of the chronic toxicity study by Fraser (1962)." Additionally, Rhodia estimates that current uses of nisin in the United States are at no more than 5% of the existing ADI level, based on industry adoption of nisin for currently approved uses.

The independent expert panel also observed in its report that "The safety of nisin is supported by a long history of safe use in foods. Nisin is naturally occurring in cheddar and American style cheeses made with traditional Lactococcus lactis starter cultures, with levels of 400 to 1200 IU/gram of cheese confirmed by various researchers. Nisin is fully hydrolyzed by standard enzymes of the upper stomach (trypsin, pepsin). A review paper (see Attachment 2: "Bacteriocins: Safe, Non-Antibiotic Food Preservatives", J. Cleveland, T. Montville, I. Nez, M. Tchikindas, submitted for Publication Aug, 2000) summarizes the history of use in foods, research on mechanisms, and evaluates

the safety and suitability in foods." A copy of the referenced review paper is included in the Appendix hereto.

The independent expert panel also stated that "There are extensive data, including numerous published reports, to support the safety of nisin, the only bacteriocin approved for use in the U.S., when used in foods as an antimicrobial. The studies include acute, subchronic, chronic, reproduction, sensitization, and cross-resistance and are cited in Attachment 2."

Finally, prior to concluding that nisin is safe for the proposed uses, the independent expert panel stated that "Nisin is the only bacteriocin approved in the U.S. for use as an antimicrobial in food. The safety of nisin is based on extensive studies in animals and is supported by a history of safe use."

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

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

Indeed, in reaching this conclusion during its critical evaluation, the independent expert panel noted in its report that "The committee raised the concern that nisin may inactivate or delay the growth of competitive indigenous microflora and, therefore, increase the risk of *L. monocytogenes*. A literature survey showed that the presence of nisin in a food product did not increase the potential for *L. monocytogenes* to reach levels to cause illness because the indigenous background microflora was inhibited.

"Data from multiple plant and laboratory studies conducted by Rhodia supports this conclusion. The presence of nisin in refrigerated hot dogs had little or no effect on the growth of the indigenous microflora and did not delay spoilage."

(C) Expert Concensus for GRAS Determination:

By way of background, in its report the independent expert panel noted that "Nisin, a lantibiotic bacteriocin, was discovered in 1928. Bacteriocins have been directly added to foods to protect against pathogenic bacteria, especially *Clostridium* and *Listeria.* Nisin is an effective inhibitor of the outgrowth of *C. botulinum* spores in cheese (Wessels, et al, 1998) and is approved for this use in the United States (21 CFR 184.1538). Nisin is effectively used in various food systems including cottage and ricotta cheeses, skim milk, bologna-type sausage, lean beef, and kimchi. Nisin is approved for food use in at least 12 countries."

As mentioned previously, the independent expert panel also stated that "There are extensive data, including numerous published reports, to support the safety of nisin, the only bacteriocin approved for use in the U.S., when used in foods as an antimicrobial. The studies include acute, subchronic, chronic, reproduction, sensitization, and cross-resistance and are cited in Attachment 2."

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 nisin proposed

herein. Indeed, the independent expert panel indicated a consensus of common knowledge of safety of the proposed uses of nisin 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 concluded that nisin, 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 nisin is safe for use as an antimicrobial agent in certain foods and is included in Attachment 2.

"The members of the Expert Panel further concluded that nisin, 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 on cooked meat and poultry products as specified herein."

Nisin
SELF GRAS DETERMINATION

APPENDIX

Rhodia

NISIN GRAS NOTICE APPENDIX

INDEX TO INCLUDED ITEMS

[redacted]

INDEPENDENT GENERALLY RECOGNIZED AS SAFE DETERMINATION OF

NISIN

(IN CERTAIN COOKED MEAT AND POULTRY PRODUCTS)

OCTOBER 2000

INDEPENDENT GRAS DETERMINATION OF NISIN

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 nisin for use as an anti-microbial agent on 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 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 nisin 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 anti-microbial properties, manufacture and processing, stability, conditions of anticipated use, estimated daily intakes resulting from these uses, and safety of nisin. This information was consolidated by Rhodia in a document attached as Appendix 2 (the dossier). The Expert Panel independently and critically evaluated the 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 nisin, 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

Certain anti-microbial substances produced by bacteria are termed bacteriocins. They have been defined as ribosomally synthesized proteinaceous compounds that inhibit or inactivate closely related bacteria (Klaenhammer, 1993). Bacteriocins are clearly distinguishable from antibiotics that are used clinically. The mechanism by which bacteriocins inactivate bacteria is by causing formation of pores in the cell membrane of the bacteria. The spectrum of activity of bacteriocins is usually narrow and limited to Gram positive pathogens. Cross-resistance of antibiotics and bacteriocins has not been demonstrated. Bacterial resistance to bacteriocins has been observed in media and in foods, but this occurs at relatively low frequency and generally at relatively high levels of the bacteriocins. Since bacteriocins are produced by beneficial lactic acid bacteria in many foods, they are considered non-toxic and safe for human consumption. Most bacteriocins, including nisin, are produced by lactic acid bacteria commonly isolated from a variety of

foods, including dairy products and meat. Bacteriocins have been consumed for centuries in dairy products and many other foods.

Nisin, a lantibiotic bacteriocin, was discovered in 1928. It was first intentionally added to foods in 1951. Nisin was recognized by FAO/WHO as an efficacious food preservative in 1969. Its structure and biochemical properties were elucidated in the 1970's and 1980's. Nisin was granted GRAS status by the U.S. Food and Drug Administration (FDA) for use in process cheese in 1988, and it has being evaluated for use in a variety of foods.

Bacteriocins have been directly added to foods to protect against pathogenic bacteria, especially *Bacillus cereus*, toxigenic *Clostridium* species, and *Listeria monocytogenes*. Nisin is an effective inhibitor of the outgrowth of *C. botulinum* spores in process cheese and is approved for this use in the United States (21 CFR 184.1538). Internationally, nisin is effectively used in various food systems including cottage and ricotta cheeses, skim milk, bologna-type sausage, lean beef, and kimchi. Nisin is approved for food use in at least 12 countries.

The committee raised the concern that nisin may inactivate or delay the growth of competitive indigenous microflora and, therefore, increase the risk of *L. monocytogenes*. A literature survey showed that the presence of nisin in a food product did not increase the potential for *L. monocytogenes* to reach levels to cause illness because the indigenous background microflora was inhibited.

Data from multiple plant and laboratory studies conducted by Rhodia supports this conclusion. The presence of nisin in refrigerated hot dogs had little or no effect on the growth of the indigenous microflora and did not delay spoilage.

4. Characteristics of Nisin

Nisin is a small antimicrobial peptide (MW: 3553 Da) produced by certain strains of *Lactococcus lactis spp lactis*, a common lactic acid producing bacterium. It has been shown to possess antimicrobial properties against select Gram positive bacteria. Nisin is the common and usual name. The Chemical Abstract Service (CAS) registry number is 1414-45-5. The empirical formula is $C_{143}H_{230}N_{42}O_{37}S_7$.

5. Manufacture

Nisin 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.

A fermentation medium including yeast extract, corn steep liquor, soy protein peptone and starch is heat-treated in high grade stainless steel fermentation tanks to achieve sterilization. The medium is cooled to an appropriate incubation temperature and inoculated with a pure culture of nisin-producing *Lactococcus lactis spp lactis*. The fermentation is allowed to progress under controlled time and temperature conditions until optimum nisin production has occurred. At the appropriate time, the fermentation is

stopped by heat treatment and processes, which may include foaming and sodium chloride precipitation under acid conditions, concentrate the nisin produced. The nisin concentrate is then reheated and dried at 140°F (60°C) for four hours, followed by standardization with sodium chloride to achieve a minimum of 1, 000, 000 IU nisin activity per gram.

Nisin conforms to the specification for "nisin preparation" set forth by the FDA in 21 CFR 184.1538 and meets the specifications that follow when it is tested as described in "Specifications for Identity and Purity of Some Antibiotics," WHO/FAO Nutrition Meeting Report Series, No. 45 A, 1969, which is incorporated by reference.

Nisin content, not less than 900 IU/mg
Arsenic, not more than 1 ppm
Lead, not more than 2 ppm
Zinc, not more than 25 ppm
Copper; zinc plus copper, not more than 50 ppm
Total plate count, not more than 10/gram
Escherichia coli, absent in 10 grams
Salmonella, absent in 10 grams
Coagulase positive staphylococci, absent in 10 grams.

In compliance with 21 CFR 184.1538, nisin concentrate is standardized with GRAS food substances to produce an activity level of not less than 1 million IU of nisin per gram as measured by the British Standards Institution Methods, "Methods for the Estimation and Differentiation of Nisin in Processed Cheese," BS 4020 (1974), which is incorporated by reference.

The analytical data from five batches (cf. Appendix 2) indicate that food grade specifications can be consistently met.

6. Uses

Nisin is proposed for use in casings for frankfurters at concentration of 3.15-mg nisin/lb frankfurter, approximately 6.9 mg nisin/kg food, and in cooked meat and poultry products sold ready-to-eat at 2.5 mg nisin/lb of cooked meat or poultry product, approximately 5.5 mg nisin/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 0.58 mg/person/day at the mean to 1.42 mg/person/day at the 95thpercentile of intake. On a per-capita basis, intakes for the U.S. population ranged from 0.24 mg/person/day at the mean to 1.04mg/person/day at the 95th percentile.

Intake estimates based on individual survey days (i.e., not two-day average) ranged from 0.24 mg/person/day (mean per-capita) to 1.38 mg/person/day at the 95th percentile per-

capita intake. On a per-user basis, intake estimates ranged from a mean of 0.94 mg/person/day to 2.24 mg/person/day at the 95th percentile of intake.

8. Safety

The FDA in 1988 critically evaluated available information on nisin, confirmed the GRAS status of nisin, and reported that nisin is safe for human consumption at an Acceptable Daily Intake (ADI) of 2.94 mg/per/day. The ADI was based on a critical re-evaluation of the chronic toxicity study by Fraser (1962).

There are extensive data, including numerous published reports, to support the safety of nisin, the only bacteriocin approved for use in the U.S., when used in foods as an anti-microbial. The studies include acute, subchronic, chronic, reproduction, sensitization, and cross-resistance and are cited in the dossier (Appendix 2). The safety of nisin is also supported by a long history of safe use in foods.

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

Summary and Conclusions

Nisin is the only bacteriocin approved in the U S for use as an anti-microbial in food The safety of nisin is based on extensive studies in animals and is supported by a history of safe use

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 nisin, when produced in accordance with current Good Manufacturing Practice and meeting appropriate food grade specifications, is safe for use as an anti-microbial agent in certain foods. This conclusion is consistent with the opinion of the FDA that nisin is safe for use as an antimicrobial agent in certain foods

The members of the Expert Panel further concluded that nisin, 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 anti-microbial on cooked meat and poultry products as specified herein.

Joseph F Borzelleca, Ph D
Professor, Pharmacology & Toxicology
Medical College of Virginia

Eric A Johnson, Sc.D
Professor, Microbiology
University of Wisconsin

John G. Cerveny
Consultant, Microbiology and Food Safety

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ESTIMATED DIETARY INTAKE OF NISIN 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
Suite 1100
Washington, DC 20036

September 12, 2000

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

I. INTRODUCTION

At the request of Rhodia, Inc. (Rhodia), Novigen Sciences, Inc. (Novigen) has estimated the dietary intake of nisin by the U.S. population and selected population subgroups in support of a self-assessment of GRAS status. Nisin is proposed for use in casings for frankfurters at concentrations of 3.15 mg nisin/lb frankfurter (approximately 6.9 mg nisin/kg food) and in cooked meats sold ready-to-eat at 2.5 mg nisin/lb cooked meat (approximately 5.5 mg nisin/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 0.58 mg/person/day at the mean to 1.42 mg/person/day at the 95th percentile of intake. On a per-capita basis, intakes for the U.S. population ranged from 0.24 mg/person/day at the mean to 1.04 mg/person/day at the 95th percentile.

Intake estimates based on individual survey days (i.e., not two-day average) ranged from 0.24 mg/person/day (mean per-capita) to 1.38 mg/person/day at the 95th percentile per-capita intake. On a per-user basis, intake estimates ranged from a mean of 0.94 mg/person/day to 2.24 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 nisin 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 nisin was based on the proposed use levels of 3.15 mg/lb frankfurter and 2.5 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 nisin. Although the use of nisin for frankfurters is limited to the hot dog casing, it was assumed that all nisin would be transferred to the hot dog and that no nisin would be lost during cooking.

III. RESULTS

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

IV. REFERENCE

U.S. Department of Agriculture (USDA), Agriculture Research Service. 1998. CSFII/DHKS Data Set and Documentation: The 1994-96 Continuing Survey of Food Intakes by Individuals and the 1994-96 Diet and Health Knowledge Survey. CD-ROM: PB98-500457. Springfield, VA: National Technical Information Service.

TABLE 1

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

	2-DAY AVERAGE INTAKE		PERSON-DAY INTAKE	
	PER CAPITA	PER USER	PER-CAPITA	PER USER
Mean	0.24	0.58	0.24	0.94
90th percentile	0.75	1.09	0.91	1.67
95th percentile	1.04	1.42	1.38	2.24

APPENDIX

PROPOSED FOOD USES INCLUDED IN THE ESTIMATED INTAKE OF NISIN

22431000 Pork roll, cured, fried
24198640 Chicken, chicken roll, roasted, light meat
24198650 Chicken, chicken roll, roasted, dark meat
24198660 Chicken, chicken roll, roasted, NS as to light or dark meat
24204000 Turkey, roll, roasted
25210110 Frankfurter, wiener, or hot dog, NFS
25210120 Frankfurter or hot dog, breaded, baked
25210150 Frankfurter or hot dog, cheese-filled
25210160 Frankfurter or hot dog, bacon and cheese-filled
25210170 Frankfurter or hot dog, chili-filled
25210210 Frankfurter or hot dog, beef
25210220 Frankfurter or hot dog, beef and pork
25210230 Frankfurter or hot dog, beef and pork, lowfat
25210250 Frankfurter or hot dog, meat and poultry, fat free
25210280 Frankfurter or hot dog, meat and poultry
25210310 Frankfurter or hot dog, chicken
25210410 Frankfurter or hot dog, turkey
25210510 Frankfurter or hot dog, low salt
25210610 Frankfurter or hot dog, beef, lowfat
25210700 Frankfurter or hot dog, meat & poultry, lowfat
25220010 Cold cuts, NFS
25220390 Bologna, beef, lowfat
25220400 Bologna, pork and beef
25220410 Bologna, NFS
25220420 Bologna, Lebanon
25220430 Bologna, beef
25220440 Bologna, turkey
25220450 Bologna ring, smoked
25220460 Bologna, pork
25220470 Bologna, beef, lower sodium
25220480 Bologna, chicken, beef, and pork
25220490 Bologna, with cheese
25220500 Bologna, beef and pork, lowfat
25220510 Capicola
25221500 Salami, NFS
25221510 Salami, soft, cooked
25221530 Salami, beef
25230110 Luncheon meat, NFS
25230210 Ham, sliced, prepackaged or deli, luncheon meat
25230220 Ham, sliced, low salt, prepackaged or deli, luncheon meat
25230230 Ham, sliced, extra lean, prepackaged or deli, luncheon meat
25230310 Chicken or turkey loaf, prepackaged or deli, luncheon meat
25230410 Ham loaf, luncheon meat
25230430 Ham and cheese loaf
25230450 Honey loaf
25230510 Ham, luncheon meat, chopped, minced, pressed, spiced, not canned
25230520 Ham, luncheon meat, chopped, minced, pressed, spiced, lowfat, not canned
25230560 Liverwurst
25230610 Luncheon loaf (olive, pickle, or pimiento)
25230710 Sandwich loaf, luncheon meat
25230790 Turkey ham, sliced, extra lean, prepackaged or deli, luncheon meat

000028

5230800 Turkey ham
25230810 Veal loaf
25230820 Turkey pastrami
25230840 Turkey salami
25230900 Turkey or chicken breast, prepackaged or deli, luncheon meat
25231110 Beef, sliced, prepackaged or deli, luncheon meat
25231150 Corned beef, pressed
27113200 Creamed chipped or dried beef
27120210 Frankfurter or hot dog, with chili, no bun
27120250 Frankfurters or hot dogs with tomato-based sauce (mixture)
27220080 Ham croquette
27420040 Frankfurters or hot dogs and sauerkraut (mixture)
27460490 Julienne salad (meat, cheese, eggs, vegetables), no dressing
27460510 Antipasto with ham, fish, cheese, vegetables
27500100 Meat sandwich, NFS
27510000 Beef sandwich, NFS
27510910 Corned beef sandwich
27510950 Reuben sandwich (corned beef sandwich with sauerkraut and cheese), with spread
27511010 Pastrami sandwich
27513010 Roast beef sandwich
27513020 Roast beef sandwich, with gravy
27513040 Roast beef submarine sandwich, on roll, with lettuce, tomato and spread
27513050 Roast beef sandwich with cheese
27513060 Roast beef sandwich with bacon and cheese sauce
27513070 Roast beef submarine sandwich, on roll, au jus
27516010 Gyro sandwich (pita bread, beef, lamb, onion, condiments), with tomato and spread
27520250 Ham on biscuit
27520300 Ham sandwich, with spread
27520310 Ham sandwich with lettuce and spread
27520320 Ham and cheese sandwich, with lettuce and spread
27520330 Ham and egg sandwich
27520350 Ham and cheese sandwich, with spread, grilled
27520360 Ham and cheese sandwich, on bun, with lettuce and spread
27520370 Hot ham and cheese sandwich, on bun
27520380 Ham and cheese on English muffin
27520390 Ham and cheese submarine sandwich, on multigrain roll, with lettuce, tomato and spread
27520540 Ham and tomato club sandwich, with lettuce and spread
27540110 Chicken sandwich, with spread
27540310 Turkey sandwich, with spread
27540330 Turkey sandwich, with gravy
27540350 Turkey submarine sandwich, on roll, with cheese, lettuce, tomato and spread
27560000 Luncheon meat sandwich, NFS, with spread
27560110 Bologna sandwich, with spread
27560120 Bologna and cheese sandwich, with spread
27560300 Corn dog (frankfurter or hot dog with cornbread coating)
27560310 Corny dog, with chili, on bun
27560320 Frankfurter or hot dog, plain, on bun
27560330 Frankfurter or hot dog, with cheese, plain, on bun
27560340 Frankfurter or hot dog, with catsup and/or mustard, on bun
27560350 Pig in a blanket (frankfurter or hot dog wrapped in dough)
27560360 Frankfurter or hot dog, with chili, on bun
27560370 Frankfurter or hot dog with chili and cheese, on bun

27560400 Chicken frankfurter or hot dog, plain, on bun
27560510 Salami sandwich, with spread
27560910 Submarine, cold cut sandwich, on bun, with lettuce
32105030 Egg omelet or scrambled egg, with ham or bacon
32105060 Egg omelet or scrambled egg, with peppers, onion, and ham
32105080 Egg omelet or scrambled egg, with cheese and ham or bacon
32105085 Egg omelet or scrambled egg, with cheese, ham or bacon, and tomatoes
32202010 Egg, cheese, and ham on English muffin
32202020 Egg, cheese, and ham on biscuit
32202110 Egg and ham on biscuit
41206030 Beans and franks
58127210 Croissant, filled with ham and cheese
58127310 Croissant with ham, egg, and cheese
58132710 Spaghetti in tomato sauce w/frankfurters
58132713 Pasta in tomato sauce w/frankfurters, canned
58145160 Macaroni or noodles with cheese and frankfurters or hot dogs

Nisin
SELF GRAS DETERMINATION

Rhodia

0630

000031

NOVASIN™ (nisin)

Description and Nomenclature:

Nisin is a small antimicrobial peptide (molecular weight of 3,510) produced by certain strains of *Lactococcus lactis* spp. *lactis*, a common lactic acid producing bacterium. It has been shown to possess certain antimicrobial properties against select Gram positive bacteria.

A. Common or Usual Name:

Nisin

B. Chemical Name:

None

C. Chemical Abstract Service (CAS) registry number:

414-45-5

D. Empirical formula:

$C_{143}H_{230}N_{42}O_{37}S_7$

E. Structural Formula:

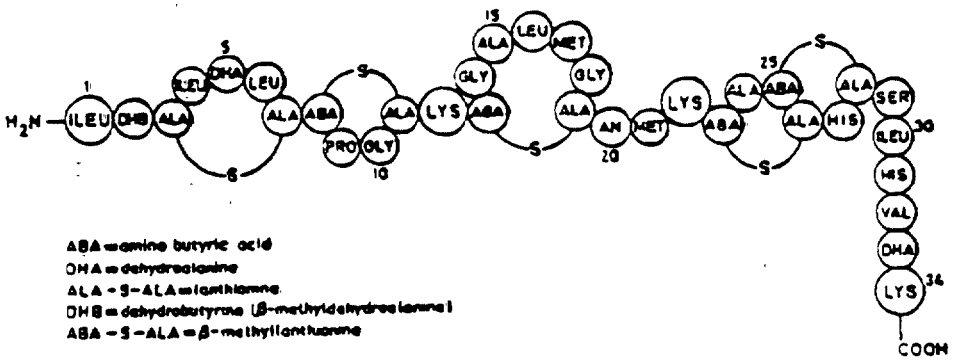


Fig. 1. The structure of nisin.

Journal of the Society of Dairy Technology, Vol. 43, No. 3, August 1990

F. Specifications for food grade material:

As used today, nisin is only commercially available as a standardized blend (or preparation) of the purified nisin peptide combined with an appropriate diluent such as sodium chloride or nonfat milk powder. 21 CFR § 184.1538 defines nisin preparation or blend as follows:

§ 184.1538 Nisin preparation.

(a) Nisin preparation is derived from pure culture fermentations of certain strains of *Streptococcus lactis* Lancefield Group N. Nisin preparation contains nisin (CAS Reg. No. 1414-45-5), a group of related peptides with antibiotic activity.

(b) The ingredient is a concentrate or dry material that meets the specifications that follow when it is tested as described in "Specifications for Identity and Purity of Some Antibiotics," World Health Organization, FAO Nutrition Meeting Report Series, No. 45A, 1969, which is incorporated by reference. Copies are available from the Dockets Management Branch (HFA-305), Food and Drug Administration, rm. 1-23, 12420 Parklawn Dr., Rockville, MD 20857, or available for inspection at the Office of the Federal Register, 800 North Capitol Street, NW., suite 700, Washington, DC 20408.

(1) Nisin content, not less than 900 international units per milligram.

(2) Arsenic, not more than 1 part per million.

(3) Lead, not more than 2 parts per million.

(4) Zinc, not more than 25 parts per million.

(5) Copper, zinc plus copper not more than 50 parts per million.

(6) Total plate count, not more than 10 per gram.

(7) Escherichia coli, absent in 10 grams.

(8) Salmonella, absent in 10 grams.

(9) Coagulase positive staphylococci, absent in 10 grams.

(c) The ingredient is used as an antimicrobial agent as defined in §170.3(o)(2) of this chapter to inhibit the outgrowth of *Clostridium botulinum* spores and toxin formation in pasteurized cheese spreads and pasteurized process cheese spreads listed in §133.175; pasteurized cheese spread with fruits, vegetables, or meats as defined in §133.176; pasteurized process cheese spread as defined in §133.179; pasteurized process cheese spread with fruits, vegetables, or meats as defined in §133.180 of this chapter.

(d) The ingredient is used at levels not to exceed good manufacturing practice in accordance with §184.1(b)(1) of this chapter. The current good manufacturing practice level is the quantity of the ingredient that delivers a maximum of 250 parts per million of nisin in the finished product as determined by the British Standards Institution Methods, "Methods for the Estimation and Differentiation of Nisin in Processed Cheese," BS 4020 (1974), which is incorporated by reference.

Copies are available from the Dockets Management Branch (HFA-305), Food and Drug Administration, rm. 1-23, 12420 Parklawn Dr., Rockville, MD 20857, or available for inspection at the Office of the Federal Register, 800 North Capitol Street, NW., suite 700, Washington, DC 20408.

Included (as Appendix __) are the specifications of Novasin™ which conform to the current CFR and all monograph requirements of the Food Chemicals Codex, 4th Edition for activity, arsenic and lead levels; AOAC, 16th edition methods for moisture and pH; and the Joint FAO/WHO Expert Committee on Food Additives, FAO Nutrition Meetings Report Series, No. 45A (1969) for zinc and copper levels. The methods used for microbiological specifications are FDA/BAM.

G. Quantitative compositions:

As mentioned above (Section F), nisin is only commercially available to the food industry as a standardized blend (or preparation). In compliance with 21 CFR § 184.1538, Novasin™ is a nisin concentrate standardized with sodium chloride to produce an activity level of not less than 1 million IU of nisin per gram as measured by the British Standards Institution Methods, "Methods for the Estimation and Differentiation of Nisin in Processed Cheese," BS4020 (1974), which is incorporated by reference.

1. Formula and Product label:

Sodium chloride, nisin (contains not less than 1 million IU of nisin/gram).

H. Manufacturing Process:

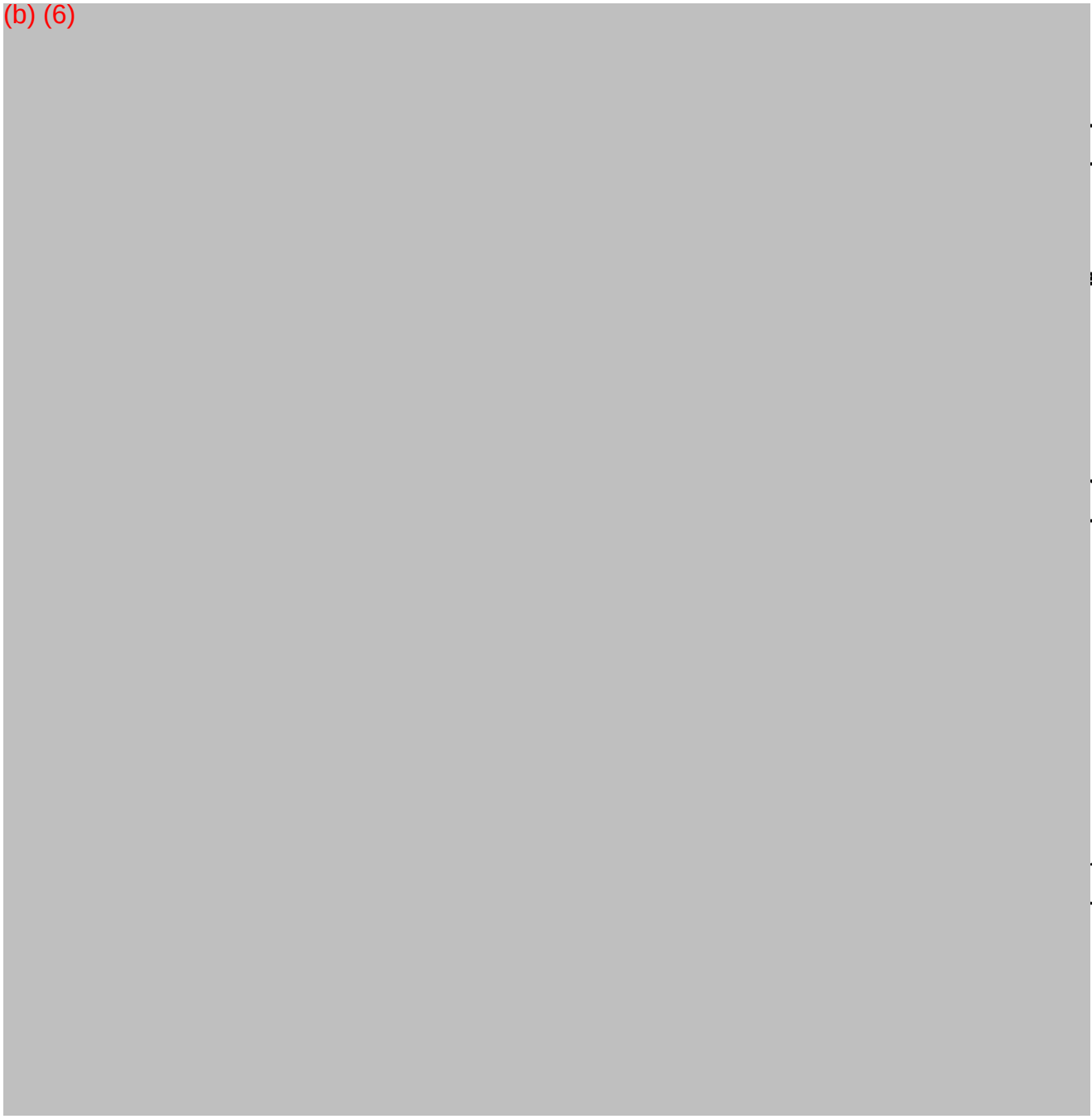
At present, all Novasin™ made for Rhodia is manufactured by Zhejiang Tiantai Silver Elephant Biochemical Plant, 22-1 Fengze Road, Tiantai, Zhejiang Province, P.R. China in compliance with Good Manufacturing Practices specified in 21 CFR, part 110, the Food Chemical Codex, Fourth Edition and any amendments thereof.

The following is a description of the manufacturing process of Novasin™ (Rhodia's trademarked name for a nisin blend or preparation).

A fermentation medium including yeast extract, corn steep liquor, soy protein peptone and starch is heat-treated in high-grade stainless steel fermentation tanks to achieve sterilization. The medium is cooled to an appropriate incubation temperature and inoculated with a pure culture of nisin-producing *Lactococcus lactis* spp *lactis*. The fermentation is allowed to progress under controlled time and temperature conditions until optimum nisin production has occurred. At the appropriate time, the fermentation is stopped by heat treatment and the nisin produced is concentrated by processes, which may include foaming and sodium chloride precipitation under acid conditions.

The nisin concentrate is then reheated and dried at 140°F for four hours, followed by standardization with sodium chloride to achieve a minimum of 1,000,000 IU nisin activity per gram and packaged as Novasin™ in 550 gram quantities in plastic bottles.

(b) (6)



CONFIDENTIAL

Elfstrum, Jim

From: Ming, Xintian
Sent: Tuesday, September 12, 2000 11:50 AM
To: Elfstrum, Jim
Cc: King, Bill
Subject: Novasin GRAS file

Jim,

Send you the analytical data of Novasin activity assay of 5 random lots, please let me know if you need any thing more for the GRAS filing.

Nisin activity assay of Novasin product

Lot number	Activity IU/mg
991222	1000
991230	1000
20000202	1000
20000124	1000
20000108	1000

Xintian

Rhodia

NOVASIN™

POWDERED NISIN PREPARATION

Product Code: M8955K

INGREDIENTS: Sodium Chloride, Nisin.
(Contains not less than 1 million IU Nisin/gram.)

MANUFACTURED for Rhodia in China

Rhodia

Rhodia Inc., Madison, WI 53701 USA

Lot No:

Quantity: 550 g. (19.38 oz.)

R9019

000039

0038

Pages 000040 - 000055 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000056 - 000094 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

SUBMISSION END

[redacted]

AM

ROBERT H. SINDT
ATTORNEY AT LAW

1850 M Street, N.W., Suite 400
Washington, D.C. 20036
Phone 202-466-4500 • Fax 202-466-5777 • E-mail rsindt@krooth.com

February 9, 2001

Dr. Negash Belay
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: Nisin GRAS Notice (GRN) No. 000065

Dear Dr. Belay:

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. 000065 filed on behalf of Rhodia, Inc. relating to specified uses of nisin. Specifically, this letter is intended to address and clarify matters you and your colleagues raised during the call regarding GRN No. 000065.

First, you noted that on the top of page 0006, the substances specified for standardizing nisin concentrate are inconsistent with other descriptions in GRN No. 000065 regarding substances used for standardizing nisin concentrate. This was an inadvertent inconsistency made in preparing the notice. The statement on page 0006 relating to standardizing "with sodium chloride and yeast extract or nonfat milk solids" should have read standardizing "with sodium chloride". Please note this correction in your review.

Next, you noted that a commercial product specification sheet on page 0036 contains certain inconsistent specifications and omits other specifications that are listed for nisin preparation at 21 CFR 184.1538, and for which the balance of the notice consistently indicates that the substance will be in compliance with such regulation specifications. Page 0036 does inadvertently misstate certain specifications and does inadvertently omit others that should be included. Consequently, attached please find a corrected and complete commercial specification sheet that is consistent both with the other specification statements and references in the notice and with 21 CFR 184.1538. Therefore, we ask that you disregard any inconsistent specifications contained on page 0036 and, instead, refer to the enclosed commercial specification sheet in your review of the notice.

The above should fully respond to and clarify the matters you raised. We apologize for the inadventent inconsistencies contained in the original submission of GRN No. 000065, 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.

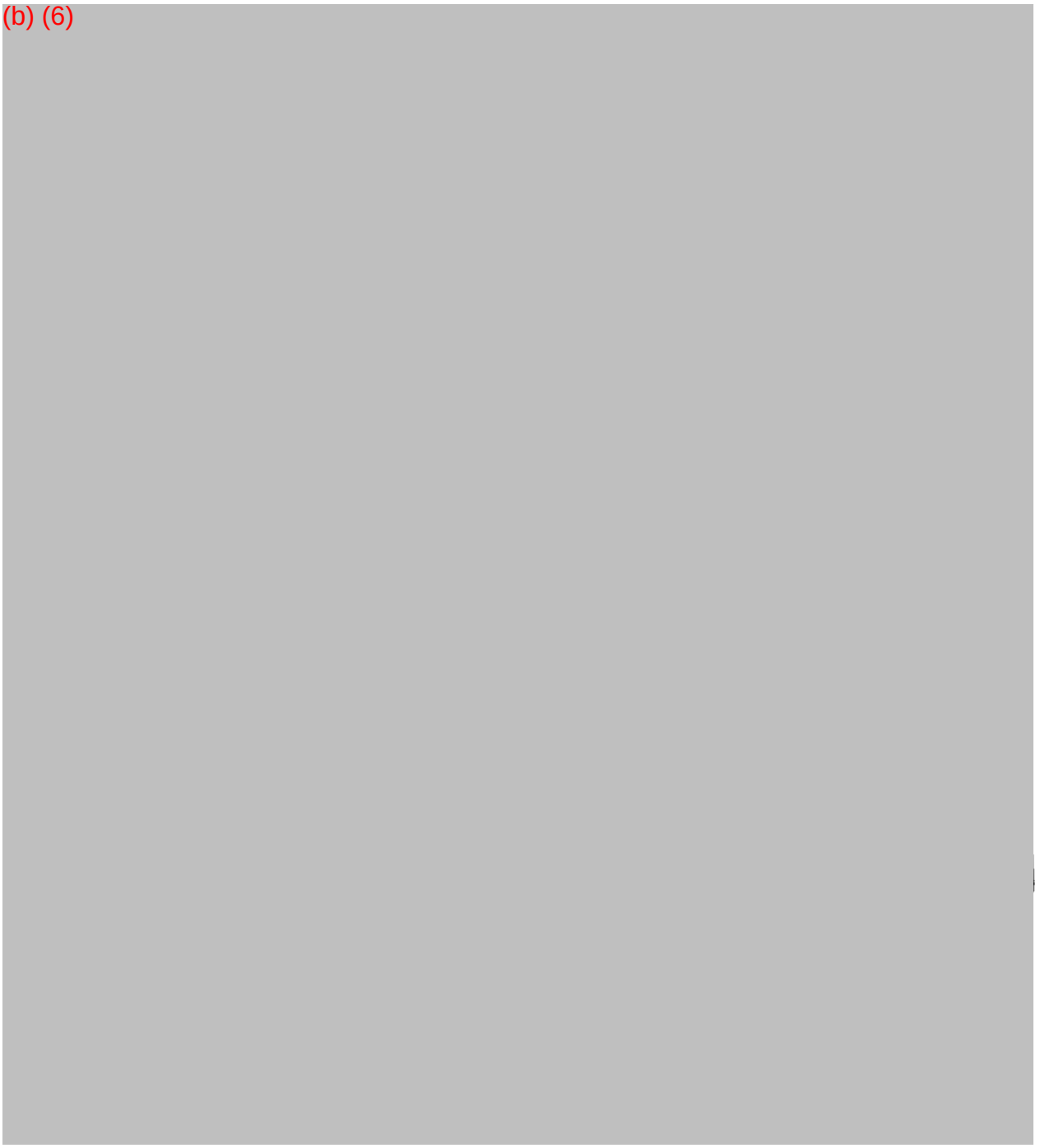
Sincerely,

Robert H. Sindt

RHS/bs

Enc.

(b) (6)



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Computer Technology Services, Inc.

000102 —
000104

AM

ROBERT H. SINDT
ATTORNEY AT LAW

1850 M Street, N.W., Suite 400
Washington, D.C. 20036
Phone 202-466-4500 • Fax 202-466-5777 • E-mail rsindt@krooth.com

March 2, 2001

Dr. Negash Belay
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: Nisin GRAS Notice (GRN) No. 000065

Dear Dr. Belay:

This will follow up and confirm the matter we discussed in our recent call regarding the above referenced GRN No. 000065 for nisin, filed on behalf of my client, Rhodia, Inc.

We discussed Rhodia's corrected commercial specification sheet, submitted to you in my letter of February 9, 2001, which contains a marking at the end of the sheet indicating it to be confidential. It is noted that the commercial specification sheet submitted with GRN 000065 (page 0036) also contained such a confidential marking. Although such a marking is appropriate for a 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 aforementioned confidentiality markings for purposes of your review of GRN No. 000065, or for valid requests FDA may receive for public production of either specification sheet.

Please let me know, if you have other questions, so that we may promptly respond to them. Thank you.

Sincerely,

Robert H. Sindt

RHS:bs

000108

AM 

1850 M Street, Suite 400, Washington, D.C., N.W.
Telecopier: (202) 466-5777
Telephone: (202) 466-4500
Email: rsindt@krooth.com

Robert H. Sindt
Attorney at Law

FAX

To:	Dr. Negash Belay, OPA/FDA	**From:**	Robert H. Sindt
Fax:	202-418-3131	**Pages:**	1
Phone:		**Date:**	03/22/01
Re:	GRN No. 000065-Nisin Uses	**CC:**	

☐ **Urgent** ☐ **For Review** ☐ **Please Comment** ☐ **Please Reply** ☐ **Please Recycle**

• **Comments: This will acknowledge and confirm your recent call where you observed that GRN No. 000065 for nisin uses specifies the name of the nisin producing cultures as "*Lactococcus lactis spp lactis*" instead of "*Lactococcus lactis* subsp. lactis", the proper name for the cultures. It is acknowledged that this error is contained in several places throughout the notice. Please make the corrections and utilize the indicated proper name for purposes of FDA's review. Thank you for bringing this matter to my attention and for providing this opportunity to correct it. Please contact me should you have other questions.**

[redacted]

000118

AM

1850 M Street, Suite 400, Washington, D.C., N.W.
Telecopier: (202) 466-5777
Telephone: (202) 466-4500
Email: rsindt@krooth.com

Robert H. Sindt
Attorney at Law

FAX

To:	Dr. Negash Belay, OPA/FDA	**From:**	Robert H. Sindt
Fax:	202-418-3131	**Pages:**	1
Phone:		**Date:**	03/27/01
Re:	GRN No. 000065-Nisin Uses	**CC:**	

☐ **Urgent** ☐ **For Review** ☐ **Please Comment** ☐ **Please Reply** ☐ **Please Recycle**

Comments: This will respond to Dr. Kahl's and your call regarding a lack of specificity of GRN-No. 000065 in identifying the strain(s) of *Lactococcus Lactis* subsp. lactis utilized in producing nisin. This will confirm that the notice applies to nisin derived from strains of microorganisms conforming in all respects to the microorganisms, as presently classified, described in 21 CFR 1538. Please utilize this clarification for purposes of your review of GRN No. 000065. I believe it addresses the lack of specificity you observed in GRN No. 000065 on this matter. Please contact me should you have other questions. Thank you.

000119

[redacted]

Reference List for Industry Submission, GRN 000065

Pages	*Author*	*Title*	*Publish Date*	*Publisher*	*BIB_Info*
000040 - 000055	NA	Methods for The estimation and differentiation of nisin in processed cheese	1966	NA	NA
000056 - 000094	Cleveland, Jennifer; Montville, Thomas; Nes, Ingolf; Tchikindas, Michael	Bacteriocins: Safe, Non-Antibiotic Food Preservatives	NA	NA	NA

NA- Not applicable