

GRAS Notice (GRN) No. 517

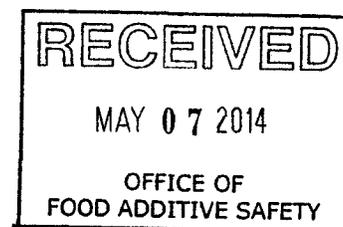
<http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/default.htm>

ORIGINAL SUBMISSION

Morgan, Lewis & Bockius LLP
1111 Pennsylvania Avenue, NW
Washington, DC 20004
Tel. 202.739.3000
Fax: 202.739.3001
www.morganlewis.com

Morgan Lewis
C O U N S E L O R S A T L A W
GRN 000517

Gary L. Yingling
Partner
202.739.5610
gyingling@morganlewis.com



May 6, 2014

VIA FEDERAL EXPRESS

Dr. Antonia Mattia
Director
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Re: GRAS Notification for the Use of Natamycin in Yogurt

Dear Dr. Mattia:

On behalf of DSM Food Specialties ("DSM"), we are submitting under cover of this letter three paper copies and one eCopy of DSM's generally recognized as safe ("GRAS") notification for its antimycotic natamycin preparation produced by *Streptomyces natalensis*. The electronic copy is provided on a virus-free CD, and is an exact copy of the paper submission. DSM has determined through scientific procedures that its natamycin produced by a submerged batch fermentation of *Streptomyces natalensis* is GRAS for use as an inhibitor against yeast and mold growth in yogurt, at levels not to exceed 5 ppm in the finished product.

Microbial spoilage of foods is a common and serious threat to food safety. Spoilage can contribute to shortages of nutritious foods, can threaten human health, and results in serious economic lost to both the producer and the consumer. Because refrigeration of food, such as yogurt, does not completely protect against spoilage, the use of preservatives such as natamycin are required to protect the quality and the safety of foods.

Pursuant to the regulatory and scientific procedures established by proposed regulation 21 C.F.R. § 170.36, this use of natamycin produced by a submerged batch fermentation of *Streptomyces*

natalensis is exempt from premarket approval requirements of the Federal Food, Drug and Cosmetic Act, because the notifier has determined that such use is GRAS.

If you have any questions regarding this notification, or require any additional information to aid in the review of DSM's conclusion, please do not hesitate to contact me via email at gyingling@morganlewis.com or by telephone, (202)739-5610.

Sincerely,

(b) (6)

Gary L. Yingling

cc: DSM Food Specialties

GRAS NOTIFICATION USE OF NATAMYCIN IN YOGURT

Submitted to:

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD
U.S.A. 20740-3835

Applicant:

DSM Food Specialties B.V.
Alexander Fleminglaan 1
2613 AX Delft
The Netherlands

Table of contents

I.	GRAS EXEMPTION CLAIM.....	4
I.A	Claim of Exemption from the Requirement for Premarket Approval Pursuant to Proposed 21 CFR § 170.36 (c) (1)	4
I.B	Name and Address of Notifier	5
I.C	Common Name of Notified Substance	6
I.D	Conditions of Intended Use in Food	6
I.E	Basis for the GRAS Determination	11
I.F	Availability of Information for FDA Review	11
II.	DETAILED INFORMATION ABOUT SOURCE AND IDENTITY OF THE SUBSTANCE.....	12
II.A	Identity	12
II.B	Physical, Chemical and Biological Properties.....	13
II.C	Product Specification.....	16
II.D	Description of the Commercial Product:	17
III.	Method of Manufacture	18
III.A	Process flow diagram.....	18
III.B	Fermentation Process	19
III.C	Purification Process.....	19
III.E	Control measures	21
IV.	COMPOSITION OF THE FINISHED PRODUCT	22
V.	DISCOVERY AND HISTORY OF USE.....	23
V.A	Discovery.....	23
V.B	History of Safe Use.....	23
V.C	Use in yogurt.....	24
VI.	USE OF NATAMYCIN TO PREVENT FUNGAL GROWTH IN FOOD	26
VII.	CURRENT REGULATORY STATUS.....	30
VII.A	Regulatory status of the proposed use of natamycin in yogurt	30
VII.B	Other approvals of natamycin with the same type of application as yogurt.....	31
VIII.	PROPOSED CONDITIONS OF USE	32
VIII.A	Food Categories and Intended Usage Levels.....	32
VIII.B	Exposure Assessment - Estimated Consumption of natamycin	33
IX.	SAFETY LITERATURE AND TOXICOLOGY STUDIES	35
IX.A	Introduction.....	35

IX.B Summary of the literature	35
IX.C Conclusion - Literature Studies	39
IX.D Toxicological studies	41
X Conclusion of the Dossier	50
ANNEXES	51

Total number of pages in dossier: 133 (excluding References)

REST OF THIS PAGE INTENTIONALLY LEFT BLANK

I. GRAS EXEMPTION CLAIM

I.A Claim of Exemption from the Requirement for Premarket Approval Pursuant to Proposed 21 CFR § 170.36 (c) (1)

The substance that is the subject of this Generally Recognized As Safe (GRAS) notification is natamycin, which is a polyene macrolide antimycotic.

Natamycin is currently codified by the US FDA under 21 CFR §172.155 for use as an antimycotic on the surface of cheese to inhibit mold spoilage in amounts not to exceed 20 ppm in the finished product, and it may be applied to grated and shredded cheese (21 CFR §133.146).

Pursuant to 21 CFR §170.30, DSM has determined through scientific procedures that its natamycin produced by a submerged batch fermentation of *Streptomyces natalensis* is GRAS for use as an inhibitor against yeast and mold growth in yogurt at levels not to exceed 5 ppm in the finished product.

I.B Name and Address of Notifier
Applicant:

DSM Food Specialties B.V.
 Alexander Fleminglaan 1
 P.O. Box 1
 2600 MA Delft
 The Netherlands
 Tel: (31) 15 279 2017
 Fax: (31) 15 279 3614

Manufacturing locations	
Fermentation	DSM Capua S.p.A. Strada Statale Appia, 46-48 81043 Capua (Caserta), Italy Tel: (39) 0823 628111 Fax: (39) 0823 628393
Recovery	DSM Food Specialties B.V. Alexander Fleminglaan 1 2600 MA Delft The Netherlands Tel: (31) 15 279 2380 Fax: (31) 15 279 3482

Person Responsible for the Dossier:

André Keuter, M.Sc.
 DSM Food Specialties B.V.
 Alexander Fleminglaan 1
 2613 AX Delft
 Tel : +31 15 2792017
 Fax : +31 15 2793614
 Email : andre.keuter@dsm.com

I.C Common Name of Notified Substance

The current name according to WHO nomenclature is Natamycin.

Synonyms: Pimaricin.

Commercial name: A registered trade name for DSM natamycin products is Delvo[®]cid.

I.D Conditions of Intended Use in Food

I.D.1. Intended use of natamycin and level of use in yogurt

Food safety is an important topic in the United States, both for the food industry and the government, as evidenced by the passage of the Food Safety and Modernization Act in 2011. Microbial spoilage of foods is a common and serious threat to food safety. Spoilage can contribute to shortages of nutritious foods, can threaten human health, and results in serious economic lost to both the producer and the consumer. Because refrigeration of food, such as yogurt, does not completely protect against spoilage, the use of preservatives such as natamycin are required to protect the quality and the safety of foods.

Yeast and molds are the main spoilage organisms found in cultured milks e.g. yogurt (Ellin Doyle 2007). Yeast and molds are not involved in the fermentation for producing yogurt. As stated by Fleet (1990), when produced under conditions of good manufacturing practice, yogurt should contain less than 10 yeast cells per gram (but preferably less than 1 cell/g) and, if refrigerated at 5 °C or less, they should not undergo spoilage by yeast. In these cases, a shelf-life of 4 weeks is expected and is limited by factors other than yeast. Yogurt which is contaminated with an initial load of 100 or more yeast cells/g will probably spoil as the yeast cells multiply. Spoilage becomes evident when the yeast population reaches 10^5 - 10^6 cells/g, and is first seen as a swelling of the yogurt package due to gas production by yeast fermentation. Occasionally, yeast colonies are seen on the under surface of the package lid. It is not uncommon to find yeast populations of 10^3 cells/g or more in retail samples of either plain or fruit yogurts (Fleet, 1990).

Natamycin is intended to be used as a preservative to prevent the growth of molds and yeasts in yogurt. Preservation studies in yogurt showed efficacy of natamycin at doses of 5-20 ppm which prevented the growth of most relevant food spoilage molds (Var et al., 2004; El-Diasty et al., 2008 and Dekker, 2012).

Var et al. (2004) investigated the effects of several levels (5, 10, 15 and 20 ppm) of natamycin and its application for use on yogurt starter bacteria, yeast and molds growth. Yogurt samples were stored at 4 ± 1 °C for up to 30 days. In the samples, the population of yogurt bacteria decreased approximately 3 log cycles in 30 days at 4 ± 1 °C.

The highest counts were observed on day 7, with the lowest number of bacteria observed on day 30. In the control samples without natamycin, molds were found to be 1.80 ± 0.10 log CFU/g and 4.27 ± 0.32 log CFU/g respectively. The numbers of yeasts in control samples on days 7, 15 and 30 were respectively 2.51 ± 0.17 , 4.59 ± 0.55 and 5.92 ± 0.46 log CFU/g.

All samples containing natamycin did not show any growth of yeast or molds. Even after 30 days of storage, no growth of yeast or molds was detected in various yogurts. Consequently, the authors concluded that natamycin would be the most suitable weak-acid preservative for use in dairy products, as it prevented completely undesirable yeast and mold growth. From a microbiological point of view, suitable storage periods for the yogurts studied could be longer than 4 months at 4 ± 1 °C.

El-Diasty et al. (2008) evaluated the shelf life and the changes occurring in the organoleptic characters of yoghurt supplemented with natamycin during the storage period. Yoghurt samples were prepared and divided into two groups; natamycin was added to the first group at the levels of 10 and 20 mg/kg, while the second group of yoghurt was prepared without natamycin and kept as a control. Yoghurt cups were stored over the experimental period of 35 days at a temperature of 4 ± 1 °C. The yoghurts were examined at the 3rd and 7th days, then weekly till the end of storage period. The treated group of yoghurt was acceptable when examined organoleptically. Yeasts and molds were not detected till the end of the storage time. On the other hand, control samples of yogurt were unacceptable when examined organoleptically and exhibited contamination with molds and yeasts. El-Diasty et al. concluded that natamycin proved to be a suitable and effective

antifungal agent that increases the shelf life of yoghurt without changing the normal organoleptic characteristics of the products in which it is used.

Natamycin is particularly effective at very low concentrations against fungi, which may produce mycotoxins and create a public health hazard. El-Diasty et al. concluded that yoghurt treated with (either 10 or 20 mg/kg) natamycin showed good characteristics upon organoleptic examination during the storage period as well as the inhibition of molds and yeast growth. This effect leads to increasing the quality and hence safety of yoghurt which is desired by manufacturers and consumers.

In the efficacy evaluation performed by Dekker (2012), natamycin and sorbate were compared on several aspects in yogurt application.

First, the effect of both preservatives on starter cultures was tested.

The results show, that natamycin has no effect at all on the starter cultures. Acidification, post acidification and stability during shelf life were all comparable to the control samples without preservatives.

Sorbate however, slowed the acidification rate during fermentation when used in concentrations higher than 0.03%, prolonging the fermentation time by several hours. Post acidification and starter culture stability during shelf life were not affected by sorbate.

The challenge test with a contaminating yeast strain showed that both sorbate (0.03%, 0.05%, 0.07%, 0.1% and 0.2%) and natamycin (5 and 10 ppm) are effective inhibitors in the concentration ranges tested. Yeast levels in both cases stayed below 50 CFU/ml during the six-week test period, which is in line with the USDA *Specifications for yogurt, nonfat yogurt and lowfat yogurt* from 2001, mentioning a microbial requirement for yeast and molds of not more than 50 per gram. The control samples, with no preservatives, showed yeast levels of approximately 100 CFU/ml within one (1) week.

Yogurt treated with either preservative even retains a somewhat higher cell count of *L.bulgaricus* than the control samples.

The natamycin and sorbate stability in the yoghurt matrix were tested during a six-week shelf life, in which concentrations were measured by HPLC every two weeks. The results show that sorbate remains stable, while natamycin slowly decreases over time.

I.D.2 Levels of Use

DSM has determined, based on the above mentioned studies that the addition of 5 ppm (5 mg/kg) natamycin to yogurt is scientifically shown to be sufficient for the prevention of food spoiling yeasts and molds in yogurt. For the yogurt application, DSM's Delvo[®]cid natamycin will be added in an appropriate concentration and mixed homogeneously with the food product (i.e. added to the milk before, during or after fermentation to produce the yogurt). This is in contrast to the use of natamycin in cheese applications where it is applied on the surface of cheese.

The rationale for the maximum dosage of 5 ppm natamycin in yogurt is to provide sufficient preservative action until the end of shelf life which is dependent of the relative condition of various yogurt products.

I.D.3 Estimated Consumption of Natamycin Based Upon Current and Intended Use

Natamycin is currently allowed for the surface treatment of cheese (21 CFR §172.155) including grated and shredded cheese (21 CFR §133.146) in amounts not to exceed 20 ppm in the finished product.

A maximum level of 5 ppm natamycin is proposed to preserve yogurt. Examples of yogurt products include plain, flavored, fruit varieties and soy yogurt. Based on the studies described above, natamycin has been shown to be effective at this proposed dose.

At the request of DSM Food Specialties, Exponent, Inc. conducted an intake assessment to estimate the total daily intake of natamycin from proposed use in yogurt products by the overall US population. The background intake of natamycin was also assessed based on Code of Federal Regulation (CFR) approved uses of natamycin in finished cheese products to estimate cumulative intake from background and proposed new uses combined. The estimated daily intake (EDI) of natamycin is based on foods reported consumed in the What We Eat in America (WWEIA) dietary component of the National Health and Nutrition Examination Surveys, (NHANES) 2007-2010.

See for details of the intake assessment Section VIII.B of this dossier.

The Exponent report is attached as Annex VIII-1.

I.E Basis for the GRAS Determination

Pursuant to 21 CFR §170.30, DSM has determined through scientific procedures that its natamycin produced by a submerged batch fermentation of *Streptomyces natalensis* is GRAS for use as an inhibitor against yeast and mold growth in yogurt at levels not to exceed 5 ppm in the finished product.

I.F Availability of Information for FDA Review

The data and information that are the basis of this GRAS determination are available for the Food and Drug Administration's (FDA) review, copies of References to this report will be sent to the FDA upon request.

Request for copies and arrangements for review of materials cited herein may be directed to:

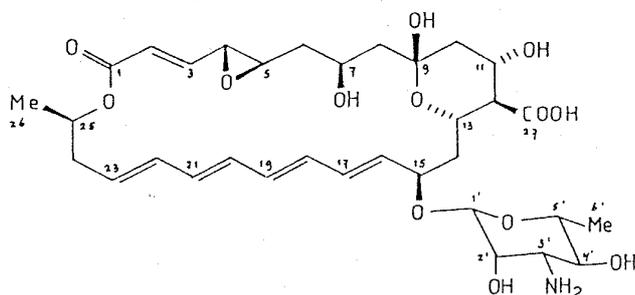
Gary L. Yingling, Esq.
Morgan, Lewis & Bockius LLP
1111 Pennsylvania Avenue, NW
Washington, DC 20004-2541
Tel : 202-739-5610
Email : gyingling@morganlewis.com

II. DETAILED INFORMATION ABOUT SOURCE AND IDENTITY OF THE SUBSTANCE

II.A Identity

Natamycin is a polyene macrolide with four conjugated double bonds and is classified as a tetraene antimycotic.

- Systematic name : Natamycin
- Synonym : Pimaricin
- Product name : Delvo[®]cid
- Chemical name: 22-(3-amino-3,6-dideoxy-β-D-mannopyranosyl)oxy-1,3,26-trihydroxy-12-methyl-10-oxo-6,11,28-trioxatricyclo [22.3.1.0^{5,7}]octacos-8,14,16,18,20-pentaene-25-carboxylic acid
- Chemical formula : C₃₃H₄₇NO₁₃
- Molecular weight : 665.7
- CAS number : 7681-93-8
- EINECS number : 231-683-5
- INS number : INS 235
- Chemical structure :



The chemical structure of natamycin can be represented by a keto form as well as a hemiketal form, with a preference for the latter. As is shown by the structural formula, a large lactone ring of 25 carbon atoms is linked to a mycosamine moiety (a pyranose). The natamycin molecule may contain up to 3 molecules of crystal water.

II.B Physical, Chemical and Biological Properties

The polyene macrolide antimycotic natamycin is produced by *Streptomyces natalensis*. The isolated material after downstream processing contains no less than 95.0% of natamycin (C₃₃H₄₇NO₁₃), calculated on the anhydrous basis. It is a white to creamy-white, almost tasteless and almost odorless, crystalline powder.

Natamycin is soluble in polar organic solvents, like glacial acetic acid and dimethylformamide, slightly soluble in methanol and practically insoluble in fatty and mineral oils and water.

The solubility of natamycin in aqueous solutions is approximately 40 ppm (Stark, 2003a).

Under influence of light or acid conditions, natamycin decomposes into inactive substances, yielding amongst others mycosamine, aponatamycin and di-natamycinolidediol for degradation products (Brik, 1976, 1981 and 1994). The acute toxicity of these products was investigated and is at an even higher dose (based on LD₅₀-values) than natamycin itself (for more details, see section IX of this dossier).

Natamycin is stable for three years when stored at temperatures below 20°C, in a dry place protected from light. As an aqueous suspension, the substance will keep its (antifungal) activity for at least six months provided the pH is kept between 6.5 and 7.5 and the suspension is stored in the dark below 20°C and kept from freezing.

Mechanism of Action

It has been shown that natamycin is active against fungi, yeasts and yeast-like organisms. As discussed in the JECFA evaluation of natamycin (Annex IX-1), natamycin is believed to bind with ergosterol, the primary sterol in fungal cell membranes. As recently demonstrated (Te Welscher 2012), natamycin inhibits growth of yeast and fungi via the immediate inhibition of amino acid and glucose transport across the plasma membrane. This is attributable to ergosterol-specific and reversible inhibition of membrane transport proteins.

Because the action of polyene antimycotics is dependent on the presence of ergosterols in the cell wall, natamycin is not active against bacteria because their cell walls do not contain ergosterols (Raab, 1973). Given the lack of effect on bacteria, use of natamycin in yogurt will not alter the probiotics in the yogurt, and will not impact the bacterial flora of the human digestive tract.

History of source strain

The original strain for the production of natamycin was isolated from a soil sample in a screening program in 1955 by researchers of the Koninklijke Gist- en Spiritus Fabrieken (KNG&SF, which later became Gist-brocades, which eventually was acquired by DSM). In view of its origin, the strain was designated *Streptomyces natalensis* and was deposited both at Northern Regional Research Laboratory (NRRL, part of the Agricultural Research Service (ARS), US Department of Agriculture) and CBS under the accession numbers NRRL 2651 and Centraal Bureau voor Schimmelcultures (CBS) 700.57 for intellectual property purposes (KNG&SF, 1957). Subsequently it was deposited at ATCC under accession number ATCC27448 for the same reasons (Struyk et al., 1975).

The identification of the original isolate as *Streptomyces natalensis* was done at the Koninklijke Nederlandsche Gist- en Spiritusfabriek NV (KNG&SF) by J. den Admirant and Dr. G.A. de Vries of the Centraal Bureau voor Schimmelcultures (1956).

A complete description of the strain was published by Shirling et al., (1972).

The International Streptomyces project has assigned *Streptomyces natalensis* to cluster 29 (*Streptomyces lydicus*) as ISP 5357 *Streptomyces natalensis* (Williams et al., 1983).

Streptomyces natalensis is classified as follows:

Kingdom	:	Procaryotae
Division	:	Bacteria
Order	:	Actinomycetales
Family	:	Streptomycetaceae
Genus	:	Streptomyces
Species	:	<i>Streptomyces lydicus</i>
Synonym	:	<i>Streptomyces natalensis</i>

Identification and safety of the source strain

A production strain PIM-10 (DS 31883) was identified by Dr. R.M. Kroppenstedt, an expert of the German Culture Collection, the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) in Braunschweig, Germany, as *Streptomyces lydicus*, in conformity with cluster 29 of *S. natalensis* status¹.

Streptomyces natalensis is an actinomycete and reproduction occurs through vegetative propagation. The organism, like most other actinomycetes, forms asexual aerial spores. However sporulation only occurs on solid media, and not during fermentation. The spores are resistant to desiccation, but not to heat.

Streptomyces natalensis is a non-pathogenic organism. Streptomyces in general are considered non-pathogenic and have their habitat in soils from all over the world. Of the more than 500 *Streptomyces* species, listed in *Sichere Biotechnologie*, only 3 are considered to be pathogenic and are listed as risk class 2; *S. somaliensis* is the only human pathogen, whereas *S. scabies* and *S. acidiscabies* are plant pathogens; all other *Streptomyces* species are listed as risk class 1 organisms.

Streptomyces natalensis CBS 700.57 and isolates derived from this strain have been used by DSM for large scale fermentation since 1960 (Brik, 1981). Present large-scale production of natamycin is carried out in the DSM Food Specialties production facility for fermentation in Capua, Italy with recovery of natamycin from the broth conducted in Delft, The Netherlands. No noticeable effects of any kind have been observed on the health of industrial workers (Raab, 1973; JECFA, 2002 and see also Section IX.I 'Irritation and Sensitization studies' in this dossier) or on the environment. The FDA confirmed in two cases that the use of natamycin did not have a significant impact on the human environment and that an environmental impact statement was not required (FDA, 1998 and 2004). The preparation of an environmental assessment (EA) or an environmental impact statement (EIS) for the current described application is categorically excluded according to 21 CFR §25.32 (k).

¹ Identification and comparison of two *Streptomyces* strains "*S. gilvosporeus*" DS35055 (00-302) and "*S. natalensis*" DS31883, Prof. Dr. R.M. Kroppenstedt, August 2000

II.C Product Specification

Description	:	A polyene macrolide antimycotic for food use containing natamycin derived from a selected strain of <i>Streptomyces natalensis</i>
Color	:	Off-white to cream colored
Melting point	:	Approx. 280°C
Purity	:	≥ 95% (on anhydrous basis)
Water	:	between 6.0% and 9.0% (crystal and free water)
Sulphated Ash	:	≤ 0.5%
Arsenic	:	≤ 1 ppm
Lead	:	≤ 2 ppm
Mercury	:	≤ 1 ppm
Total viable count	:	≤ 100 CFU/gram

The natamycin molecule may contain up to 3 molecules of crystal water.

The product specification for DSM's natamycin is in compliance with the US FDA 21 CFR §172.155, Food Chemicals Codex (FCC) 8th edition and international specifications, e.g. EU specification on Food Additives and CODEX/JECFA monograph.

The above mentioned specifications cover descriptions for:

- Product definition;
- Synonyms;
- Chemical coding (e.g. CAS and EINECS);
- Chemical formula and molecular weight;

and set limits for:

- Assay;
- Identification parameters (e.g. pH range, UV absorption);
- Purity parameters (e.g. microbiological levels and heavy metals).

The analytical method to assay natamycin is an HPLC method which is included in the FCC 8th edition and the JECFA monograph for natamycin. In Annex II.C-1, a complete overview of all specifications and requirements is shown.

II.D Description of the Commercial Product:

DSM's natamycin will be available in two liquid formulations differing in concentration of natamycin and pH and two powder formulations differing in bulking agent:

Delvo[®]cid L2

- A stable off-white liquid dispersion² consisting of 13.9% (w/w) natamycin in water, pH adjusted to 4.0 ± 0.5

Delvo[®]cid +

- A stable off-white liquid dispersion⁴ consisting of 4% (w/w) natamycin in water, pH adjusted to 4.5 ± 0.5

Delvo[®]cid XT1

- A stable off-white powder consisting of $\geq 50\%$ (w/w) natamycin with xanthan gum as bulking agent

Delvo[®]cid salt

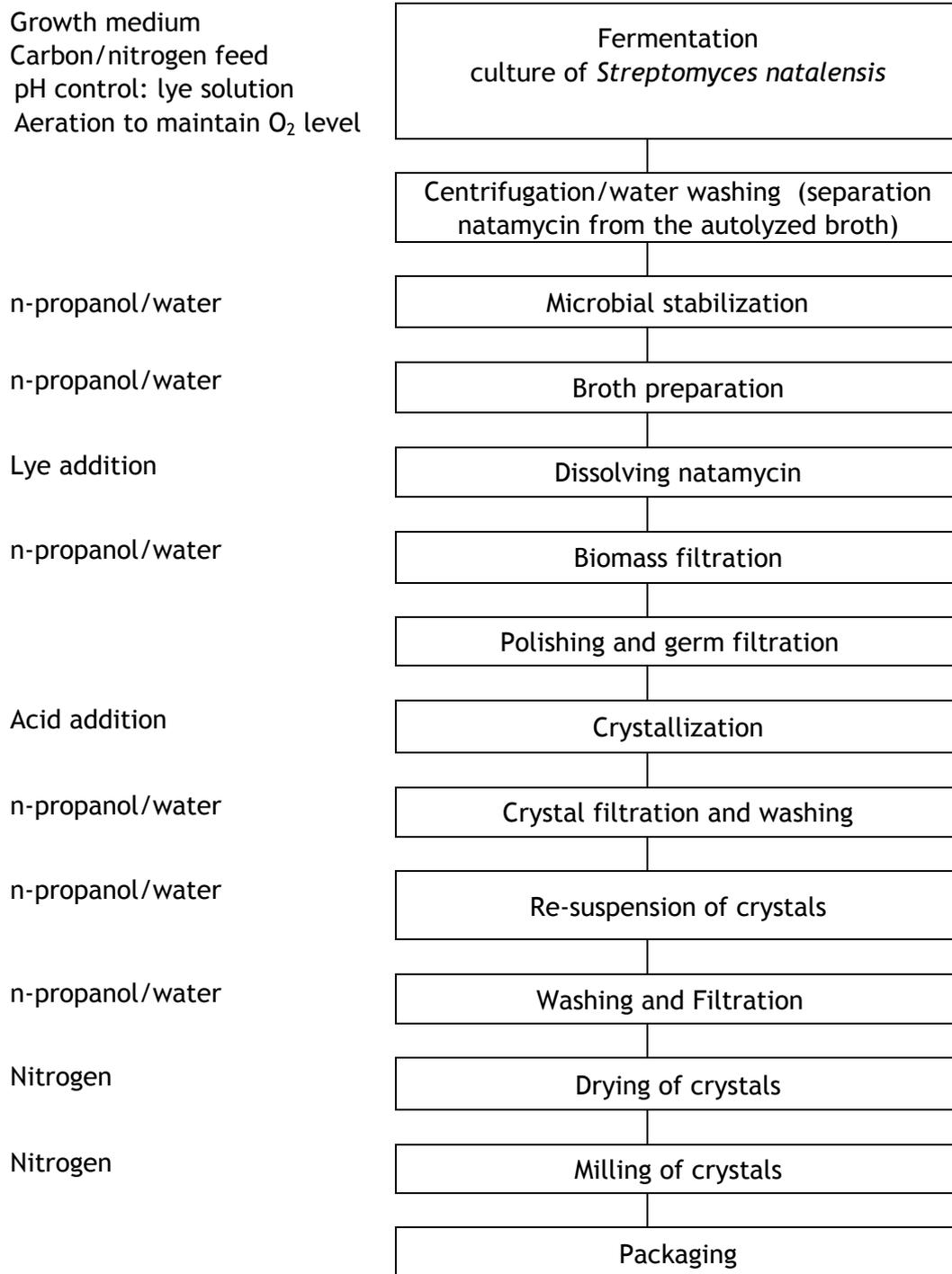
- A stable off-white powder consisting of $\geq 50\%$ (w/w) natamycin with sodium chloride as bulking agent

² liquid dispersion = a liquid of a different composition in which particles are dispersed

III. Method of Manufacture

III.A Process flow diagram

A flow chart of the natamycin production process is shown below:



The manufacturing process consists of a classical submerged batch fermentation, downstream processing and formulation of the final product (Struyk, 1975; Brik, 1981).

Natamycin powder is formulated into final liquid and powder products (see also section II.D).

All raw materials used for the fermentation, downstream processing and final formulation are food grade quality and meet predefined quality standards that are controlled by the Quality Assurance Department of DSM Food Specialties. The raw materials are suited for their intended use and help assure the safety of the product. The entire manufacturing process is performed in accordance with current Good Manufacturing Practices (cGMP) in a manufacturing process that includes a HACCP plan.

III.B Fermentation Process

Natamycin is produced by a pure culture of *Streptomyces natalensis*. The culture is continuously aerated and fed with media (C-source, inorganic salts) to support the growth of the microorganism and production of natamycin (e.g. C-source: soy product). DSM Quality standards require a strictly controlled fermentation process. The DSM fermentation factory in Capua, Italy, produces natamycin under cGMP and ISO 9001-2000 certification.

III.C Purification Process

After fermentation, the broth is transferred for further processing to the DSM facility in Delft, The Netherlands. Recovery and purification is performed in the Delft facility which achieved certification by ISO 9001-2000, ISO 14001-2004 and International FSSC 22000:2010 and operates under a system of current Good Manufacturing Practices (cGMP).

After fermentation is complete, the broth, containing the insoluble natamycin, is subject to centrifugation where the natamycin crystals are separated from most of the residual mycelium and are isolated as a suspension.

The crystals in the suspension are re-crystallized for purification purposes. The natamycin crystals are filtered and washed. The washed crystals are

then re-suspended and filtered again. The natamycin crystals are dried and milled to the final powder form and packed.

Liquid and dry product: see Sect IV, Composition of the Finished Product.

III.D General Production Controls

Technical measures

The batches of primary seed material are prepared, preserved and stored so that contamination and degeneration is avoided and genetic stability is secured. The vials are clearly labeled and strict aseptic techniques are applied during the recovery of the culture.

Only sterilized nutrient media are used for the fermentation. The fermentor is a contained, continuously fed aerobic system. Prior to inoculation, the fermentor is cleaned, rinsed and sterilized. Membrane valves, air filters and seals are regularly checked, cleaned and replaced if necessary. Only sterilized air is used in the fermentation.

The sterilized nutrient medium and the complete biomass broth are transferred aseptically to the main fermentor. For optimal production of natamycin, it is very important that hygienic conditions during the whole fermentation process are strictly controlled. Microbial contamination would immediately result in less growth of the production organism and consequently in a low yield of natamycin. In addition to the microbial hygiene, it is important to note that only food grade raw materials and processing aids are used during fermentation.

The quality of the stock culture and the strict control of parameters such as pH, temperature and aeration during fermentation are also of utmost importance for optimal natamycin production and yield.

The preparation of sterile media and the cleaning of the equipment are stated in Quality Assurance documents and are strictly followed. The manufacturing methods effectively prevent microbial contamination during fermentation. This results in a solidly established program of Good Manufacturing Practices for food within the framework of an ISO certified system. The DSM Food Specialties plant is registered with the US FDA and manufacturing is done in compliance with 21 C.F.R. §110, the Good Manufacturing Practices for Food.

III.E Control measures

Control measures are taken to assure the overall quality of the raw materials, the seed microorganism and the methods used for cell banking. Parameters that may influence the product yield have been identified according to the HACCP procedure and are checked on-line and/or off-line. The production process including fermentation and down-stream processing is monitored based on critical parameters as determined in the HACCP review. Any deviation is investigated and addressed according to the current quality system for the manufacture of natamycin.

After preparation of a new batch of primary seed material, samples are checked for identity, viability and microbial purity. If these parameters are correct, the strain is tested for production capacity. Only if the productivity and the product quality meet the required standards will the new batch of primary seed material be accepted for commercial production. Each time a vial from a certified batch of primary seed material is used for production, the viability, purity and identity of the strain is reconfirmed.

All raw materials used for the fermentation and recovery of the product are carefully chosen and suited for the intended use. Selection of proper food grade raw materials is one factor in assuring the safety of the product. The raw materials meet predefined quality standards that are controlled by the DFS Quality Assurance Department. The raw materials used for the formulation are food grade quality meeting the specifications of the Codex Alimentarius and/or Food Chemicals Codex 8th edition.

The finished product is subjected to extensive controls and complies with specifications of the US FDA, JECFA, EU, and FCC.

IV. COMPOSITION OF THE FINISHED PRODUCT

Natamycin intended for use in yogurt applications is marketed as a water-based liquid dispersion³ or as powder under the trade name Delvo[®]cid (see also section II.D).

In Table IV-1 the composition of the different finished products are shown.

Table IV-1 Composition of the finished products

Product	State	Natamycin w/w-%	pH	Xanthan w/w-%	Salt w/w-%	Water
Delvo [®] cid L2	Liquid dispersion	≥ 13.9	4.0	Approx. 0.4	Approx. 10	liquid
Delvo [®] cid +	Liquid dispersion	≥ 4	4.5	Approx. 0.4	Approx. 8	liquid
Delvo [®] cid XT1	Solid	≥ 50	N.A.	Bulking agent	N.A.	N.A.
Delvo [®] cid salt	solid	≥ 50	N.A.	N.A.	Bulking agent	N.A.

N.A. = Not Applicable

All ingredients used in commercial forms are food grade and meet the specifications of the FCC 8th edition.

³ liquid dispersion = a liquid of a different composition in which particles are dispersed

V. DISCOVERY AND HISTORY OF USE

V.A Discovery

Natamycin (pimaricin) is a polyene macrolide antimycotic substance, active against a broad variety of yeasts and molds present in nature. Because of its specific mode of action it is not active against bacteria (Hamilton-Miller, 1974; Raab, 1973).

Natamycin was discovered in the 1950's and described for the first time by (Struyck, 1958). The polyene antimycotic was first isolated from a *Streptomyces* strain derived from soil near PieterMaritzburg, a town in the province of Natal, South Africa. The source organism was subsequently named *Streptomyces natalensis*.

V.B History of Safe Use

Natamycin has a long history of safe use as an antimycotic in cheese and later in other dairy products. This bio-preservative has been produced since the 1960's in Delft, The Netherlands by DSM Food Specialties B.V. As of 1967, natamycin was approved worldwide as a food additive to be applied on the surface of (specific) cheese(s), preventing growth of unwanted molds and yeasts. In addition, it is also permitted on the surface of specific sausages in some countries.

In the United States and in Canada, the use of natamycin is allowed on shredded and grated cheese according to 21 C.F.R. §133.146 (see also section VII "Current regulatory status") since 1982. When applied to the surface of shredded cheese, natamycin is consumed with the cheese. This is a different scenario compared to application of natamycin to the surface of block cheese and cuts of cheese. The proposed use of natamycin in yogurt is the same type of application as use in grated and shredded cheese, whereby a low amount of natamycin is consumed.

Natamycin has broad-spectrum activity against yeasts and molds yet is safe for the consumer at low concentrations, has no negative effects on the quality of food products and has a prolonged working time (Stark, 2003a, b). Since its approval and subsequent use on cheese surfaces by cheese makers around the world and especially in USA and Canada on grated and shredded cheese, it is fair to say that natamycin has been applied to many tons of

cheese consumed by humans for years without any adverse effects proving a history of safe use at the applied levels.

V.C Use in yogurt

According to most national and international standards of identity, yoghurt is defined as a cultured milk product, containing various types of lactic acid bacteria, to which defined additives like fruits, starches, sugars, flavoring may be added. Generally, it is considered that the consumption of yoghurt is beneficial for human health since the lactic acid bacteria and especially *L. acidophilus* and some Bifidus strains are able to survive the passage through the digestive tract and establish an optimum gut flora in the large intestine (Fleet 1990; Ellin Doyle 2007). An essential fact, in this respect, is that the lactic acid bacteria are still viable when they reach the consumer. This implies that heat treatment at the end of the fermentation should be avoided. Thus for those who consider viable bacteria as a special asset of this product, pasteurization is not an alternative for preservation. This puts constraints on the hygiene control systems and the possible ways of preserving yoghurt to give it the quality and shelf life as required by retailers and consumers.

Several studies showed spoilage by yeast and molds in food and the presence of mycotoxins in marketed dairy products including yogurt (Scott, 1989; Fleet, 1990; Montagna, 1998; Lourens-Hattingh, 2002; Viljoen, 2003; Boor, 2006; Ellin Doyle, 2007; Ledenbach, 2009).

Natamycin has no activity against bacteria (Hamilton-Miller, 1974; Raab, 1973) and therefore will not disturb the normal process of ripening (ageing) of yogurt, but still enforces the need for a producer to maintain good manufacturing practices to prevent bacterial contamination by e.g. *E. coli* or *Salmonella* species.

Natamycin has been demonstrated to confer a biocidal effect on yeast and molds in dairy preparations (as described in Section I.D of this dossier), providing better control of microbiological shelf life and food safety risks.

The use of natamycin in yogurt is the same type of application as use in grated and shredded cheese, whereby a low amount of natamycin is consumed. Grated and shredded cheeses are produced and consumed in large quantities in the US on a daily basis. Therefore it is not expected that the consumption of natamycin due to application in yogurt will influence the

safety of use. Furthermore, it will prevent undesirable yeast and mold growth in yogurt which results in a reduction of spoilage and an improvement of food safety.

VI. USE OF NATAMYCIN TO PREVENT FUNGAL GROWTH IN FOOD

The occurrence of microbial spoilage caused by yeasts and molds, on and in food is a common phenomenon (Van Walbeek, 1968; Fleet, 1990; Ellin Doyle, 2007). Even under optimal conditions, microbial spoilage of food products will take place during storage, unless adequate prevention measures are taken. Although refrigeration of dairy products including yogurt, prolongs their shelf life, it alone does not offer complete protection from microbial spoilage. Refrigeration and in a sense, ‘Use by’ dates have been the only tools industry has to avert spoilage in yogurt.

From the literature on this subject (Stark, 2003a, 2003b, 2007), it can be concluded that fungi can produce mycotoxins, of which presently there are more than a hundred known.

Microbial spoilage of food is undesirable for a number of reasons including:

1. It can contribute to shortages of highly nutritious products;
2. It can be a hazard to human health;
3. Microbial spoilage causes measurable economic losses to the producer and consumer.

Cleaning of surfaces of mold-contaminated foodstuffs is not sufficient because it does not eliminate mycotoxins which could have migrated into the food and mycelium that has penetrated food (Holley, 1981).

For effective mold prevention, it is essential to have not only current good manufacturing practices (cGMP) implemented in the food processing plant, but also the use of safe and permissible preservatives is essential part of an overall food safety program. Due to its recognized inherent safety, natamycin has been used for many years in the US and most European countries as a surface treatment for cheeses (and cured sausages) and on grated and shredded cheese in USA and Canada. In that respect, natamycin has proven to be highly effective in controlling yeast and molds. Natamycin has very low human toxicity but is a potent fungicide with high specific activity against all common molds and yeasts known to cause food spoilage. Initial research was published by Struyk (1958) and Raab (1973). They showed that natamycin was active against a wide range of mycotic organisms such as dermatophytes, other fungi, yeast, yeast-like organisms

(including those strains pathogenic for man, animals and plants and the saprophytic varieties). This was later confirmed by the research published by Stark (2003a, 2003b and 2007) on different applications in food and plant protection.

Klis (1959) compared the in vitro activity of sorbic acid and natamycin and found the latter to have 50 - 100 times more activity. However in actual practice, it was found that when natamycin is used on cheese it exhibits 200 times more activity than potassium sorbate (de Ruig, 1985). This can easily be understood because natamycin, in contrast to sorbate, hardly penetrates or diffuses into the cheese rind. Instead, natamycin, due to its crystalline state and very low solubility in water (approximately 40 ppm) and insolubility in lipids remains on the surface of the cheese preventing attack from ubiquitous sources of mold.

Shahani, 1973, 1977 demonstrated that concentrations of natamycin, which were too low to completely stop the growth of molds, could almost completely inhibit the production of mycotoxins. No such effect, or to a much lesser level, was obtained with sorbic-, benzoic- and propionic acids. As stated before, the occurrence of mycotoxin producing molds is not limited to cheese alone. Van Walbeek (1968) for example, found mycotoxin producing molds in orange juice, ham, cheese, vegetables, apple squash, meat pie, rice, grains (used in animal feed) and Brazil nuts.

On the basis of the results of toxicity studies (see section IX of this dossier for detailed discussion) it can be concluded that natamycin can safely be ingested for long periods of time. No systemic toxicity, teratogenic or mutagenic effects were observed in animal toxicity studies or human clinical trials. To the best of our knowledge (based on experience of more than 25 years) no allergic or sensitization reactions have been observed. There is no indication that natamycin, when consumed, will give rise to the development of resistant fungi pathogenic in humans.

On theoretical grounds, it is highly unlikely that resistance will develop (Khoudokormoff, 1974) and in practice until now, no reports have come to our attention that such resistance ever has developed. This conclusion was also put forth in the 2002 JECFA evaluation of natamycin, which concluded that fungal resistance to natamycin was unlikely, given the low concentrations used and mechanism of action of the polyene antimycotics. To further evaluate this possibility, a review report on resistance and cross-resistance of natamycin was prepared by DSM Food Specialties in 2007 (Samson, 2007), and concluded the following:

Although natamycin has been used worldwide for decades both in the food industry and for pharmaceutical applications, resistance does not occur, even in cheese factories where natamycin has been used in daily practice for 30-40 years. This fact is perhaps the best proof that fungi cannot develop resistance to natamycin. However, further evidence that resistance will not occur has also been provided by many scientific studies. In addition, medical experts deem the development of resistance via ingestion from food impossible.

To substantiate these conclusions further, DSM Food Specialties organised an independent literature review on natamycin resistance. The literature review was performed by TNO, the Netherlands Organisation for Applied Scientific Research (www.tno.nl) in 2012. The search covered the literature published in the period 2000 till present (TNO, 2012). TNO concluded as follows:

Natamycin is an effective and safe food preservative with fungicidal activity and has wide regulatory status throughout the world. In addition, it is a well-recognized antifungal compound effective in curing topical fungal infections but not against systemic infections like mycoses. Because of its chemical structure, the compound has low solubility in aqueous solutions. It interacts selectively with fungal membranes resulting in changed membrane function and ultimately causing cell death.

Natamycin interacts with ergosterol in the fungal membranes resulting in obstruction of the membrane functionality causing cell death. Information on resistance against natamycin is limited available. Where mentioned, resistance is intrinsically caused by low presence of ergosterol in the fungal membranes which may be caused by mutation in the ERG3 gene that mainly seems a side effect of

acquired resistance against azoles. Recent literature reporting natamycin resistance as an issue is exceptional. Resistance is generally described as a natural trait in some strains. The mechanism of increased drug efflux in fungi in the development of resistance against polyenes is considered not likely since polyenes do not require entrance to the cell. It appears that mechanisms by which resistance can spread to other fungal strains are rather limited compared to the situation encountered in bacterial strains where horizontal gene transfer is a far more common phenomenon. Described natamycin resistance is mainly due to natural resistance and no reports have been published claiming acquired natamycin resistance in fungi due to horizontal gene transfer. Experimental evidence of fungi acquiring resistance to natamycin is not encountered so far.

The theoretical arguments are also supported further by DSM's own studies in cheese warehouses. Analysis of the fungal flora collected in cheese warehouses, in which natamycin was used for more than ten years, did not show any change of sensitivity of yeasts and molds towards natamycin when compared to organisms isolated from warehouses where natamycin had never been used (de Boer, 1977; Hoekstra, 1998 and Stark, 2003a).

Overall it can be concluded that natamycin has the following advantages:

- It is highly effective in controlling the formation of yeast and mold;
- It has an inhibitory influence on the production of mycotoxins;
- Natamycin has low toxicity to humans;
- It is absent of allergic and sensitization reactions;
- There is no indication of any development of resistance (even after many years of continuous use).

VII. CURRENT REGULATORY STATUS

Worldwide, countries permit natamycin as a food additive on cheese and cured sausages. In annex VII-1 an overview of the food additives legislation in the major countries is given.

Since its approval and subsequent use on cheese surfaces by cheese makers around the world and especially in USA and Canada on grated and shredded cheese, it is fair to say that natamycin has been applied to many tons of cheese consumed by humans for years without any adverse effects.

VII.A Regulatory status of the proposed use of natamycin in yogurt

Below, the regulatory status on the current use of natamycin in yogurt as described in the different national legislations or guidance:

USA:

The use of natamycin is codified in the U.S. Code of Federal Regulations at 21 C.F.R. §172.155 and 21 C.F.R. §133.146. In these regulations, the FDA approves natamycin for use on cheese surfaces and grated and shredded cheese at levels not to exceed 20 mg/kg in the finished cheese product.

While not an official regulatory status, the US FDA Milk Safety Team stated on January 29, 2007 that:

“The appropriate use of safe and suitable preservatives in yogurt, lowfat yogurt or nonfat yogurt will not be the basis for regulatory action by the FDA. Based on the information currently available, the US FDA is not prepared to challenge the use of natamycin as a preservative in yogurt, sour cream, cottage cheese and cottage cheese creaming mixture” (US Memorandum prepared by Milk Safety Team (HFS-626) for all Regional Food and Drug Directors in January 2007, pages 3 and 4).

The FDA also published a proposed rule in the Federal Register volume 74, number 10, pages 2443-2460, January 15, 2009, in which it was proposed to revoke the standards of identity for Lowfat yogurt, Nonfat yogurt and also to amend the standard for Yogurt, particularly to include optional ingredients such as stabilizers, emulsifiers and preservatives.

South Africa:

Natamycin is allowed in yogurt in South Africa at 10 mg/kg since 1977 as mentioned in South Africa regulation on preservatives and antioxidants, published under government notice No. R965.

The market size of yogurt in South Africa increased from 59,700 tonnes in 1998 to 181,100 tonnes in 2012 (Packaged Food: Euromonitor from trade sources/national statistics). Based on MINTEL data (January 2012 - May 2013) the percentage of yogurt products in South Africa that contain natamycin was 25.9%.

VII.B Other approvals of natamycin with the same type of application as yogurt

When applied in yogurt, the natamycin is consumed with the final product. Other applications with the same scenario of intake as yogurt have received approvals in:

Canada:

Approval was granted under the Food and Drug Regulations, C.R.C. 870, Division 16 “Food Additives: part III (N.1) “(D) natamycin applied to the surface of the cheese in an amount that does not exceed 20 parts per million or, if the cheese is grated or shredded, 10 parts per million.”.

China:

Approval was granted under regulation GB2760 Food Additives for fruit and vegetable juice (pulp), fermented wine and pastry (moon cakes) with a maximum residue level of 10 ppm.

VIII. PROPOSED CONDITIONS OF USE

VIII.A Food Categories and Intended Usage Levels

As mentioned in Section VII, natamycin is regulated in the US as a surface treatment for cheese to prevent mold and yeast growth and thereby prevent food spoilage. In these applications, the maximum dosage levels of natamycin do not exceed 20 ppm in the final product. As supported by the numerous literature sources, as outlined in section I.D., the application of natamycin in other dairy products such as yogurt has been found to be effective and beneficial by preventing spoilage of the product (see also Section VI).

In yogurt applications, natamycin is added directly to the product which is comparable with the application on grated and shredded cheese in the USA and Canada. The efficacy (as fully outlined in section I.D) was demonstrated in an efficacy study performed by DSM (Dekker, 2012) and in literature described by El-Diasty et al. (2008) and Var et al.(2004).

Based on the efficacy studies for natamycin in yogurt, DSM recommends for natamycin a maximum use level of 5 ppm.

The rationale for the maximum dosage of 5 ppm natamycin in yogurt is to provide sufficient preservative action till the end of shelf life which is dependent of the relative condition of various yogurt products.

VIII.B Exposure Assessment - Estimated Consumption of natamycin

At the request of DSM Food Specialties, Exponent, Inc. conducted an intake assessment to estimate the total daily intake of natamycin from proposed use in yogurt products by the overall US population and sub-populations (Annex VIII-1).

Natamycin is currently allowed for the surface treatment of cheese (21 CFR §172.155) including grated and shredded cheese (21 CFR §133.146) in amounts not to exceed 20 ppm in the finished product.

For the new application, Natamycin is proposed for use in yogurt products at a maximum use level of natamycin of 5 ppm. Examples of yogurt products include plain, flavored, fruit varieties and soy yogurt.

Estimated food intakes intended for natamycin from background and proposed uses were based on food consumption records collected in the WWEIA component of NHANES conducted in 2007-2008 and 2009-2010 (NHANES 2007-2010). This continuous survey uses a complex multistage probability sample designed to be representative of the civilian U.S. population (NCHS 2010, 2012). The NHANES datasets provide nationally representative nutrition and health data and prevalence estimates for nutrition and health status measures in the United States. Statistical weights are provided by the National Center for Health Statistics (NCHS) to adjust for the differential probabilities of selection.

The total estimated daily intake of natamycin from the background cheese consumption by the overall US population in units of mg/day and mg/kg-bw/day is provided in Table VIII-1.

Daily cumulative natamycin intake estimates from combined background and proposed uses by the overall US population and children and adolescent sub-groups is provided in Table VIII-2.

Table VIII-1 Background estimated 2-day average daily intake of natamycin from approved uses in non-processed cheese by the total US population; NHANES 2007-10

			Per Capita		Per User	
			Mean	90 th percentile	Mean	90 th percentile
Natamycin ¹	N-user ²	% Users				
Total US population						
mg/day	10,093	66	0.36	0.96	0.54	1.17
mg/kg-bw/day			0.006	0.016	0.009	0.020

¹ Based on maximum approved use level of natamycin at 20 ppm in non-processed cheese (21CFR§172.155).

² Un-weighted number of consumers; % user, per capita and per user estimates based on statistical weights provided by the National Center for Health Statistics (NCHS)

Table VIII-2 Cumulative estimated 2-day average daily intake of natamycin from background and proposed uses for the total US population and sub-populations (mg/kg-bw/day); NHANES 2007-10

			Per Capita		Per User	
			Mean	90 th percentile	Mean	90 th percentile
Natamycin	N-user ¹	% Users				
Total US population	10,745	70.6	0.008	0.019	0.011	0.023
Adults 19+ y	6,517	70.1	0.006	0.015	0.008	0.018
Children 1-3 y	910	76.3	0.025	0.064	0.033	0.071
Children 4-6 y	681	75.0	0.018	0.047	0.024	0.051
Children 7-12 y	1,359	74.8	0.010	0.024	0.014	0.028
Males 13-19 y	684	73.9	0.008	0.019	0.011	0.023
Females 13-19 y	642	75.4	0.006	0.018	0.009	0.020

¹ Un-weighted number of consumers; % user, per capita and per user estimates based on statistical weights provided by the National Center for Health Statistics (NCHS)

As noted in Table VIII-2, the mean intake per user, 0.01, represents approximately 3% of the Acceptable Daily Intake (ADI) limit of 0.3 mg/kg-bw/day, set forth by the global regulatory bodies CODEX/JECFA and EFSA. Further, all values of the 90th percentile for the total US population and sub-populations are well below the ADI. These levels are consistent with the global intake assessment document in the JECFA evaluation of natamycin (Annex IX-1), which stated that the average intake across Australia, Germany, New Zealand and the US ranged from 2.5 - 5% of the ADI.

IX. SAFETY LITERATURE AND TOXICOLOGY STUDIES

IX.A Introduction

In addition to existing oral exposure of humans resulting from the currently approved grated/shredded cheese application in the US, the proposed use of natamycin in yogurt will lead to additional oral exposure of humans. The oral toxicity profile, which reflects the route of human exposure when natamycin is used in yogurt and cheese, was therefore reviewed. This product safety appraisal based on a review of the ADME and toxicity studies is described below.

Reference is made to the latest safety evaluation of natamycin executed by the CODEX/JECFA (JECFA 2002) which confirmed the previously established ADI of 0.3 mg/kg bwt for natamycin, which was based on observations of gastrointestinal effects in humans. In Annex IX-1, the full safety evaluation report is included.

IX.B Summary of the literature

IX.B.1 Absorption, Distribution and Excretion

Raab states that natamycin is not absorbed from the gut in animals or humans (Raab, 1973). This absence of absorption from the gut would explain the apparent lack of systemic toxicity via the oral exposure route.

The safety of use in humans was confirmed by the studies used by the Joint FAO/WHO Expert Committee on Food and the EFSA, which used human data to establish a dietary limit and indicated in their evaluation that natamycin was not absorbed to a significant extent in the human digestive tract (JECFA, 2002, EFSA, 2009).

IX.B.2 Mutagenicity Studies

Ames test

Using two histidine-requiring *Salmonella typhimurium* mutant strains: TA 98 and TA 100 and two tryptophan-requiring strains of *Escherichia coli*, WP2uvrA and its mutant uvrA-, Khoudokormoff et al., investigated natamycin and three of its degradation products for their mutagenic potential.

Test concentrations were 0.04 to 1% of Delvo®cid, 0.5 to 0.02% natamycin, aponatamycin, dinatamycinolidediol and mycosamine HCL (5 and 10 mg/ml each). Various concentrations of nitrite were added to Delvo®cid suspensions and the mixtures tested after different times of storage at 18 °C in the dark.

In this assay, neither natamycin nor its degradation products were found to be mutagenic. The negative Ames test result was observed in all strains tested in both the absence and presence of S-9 (Khoudokormoff, 1977 and 1983). These results were confirmed by another study conducted by Verspeek-Rip, in 2002.

IX.B.3 Acute Oral Toxicity Study

Natamycin is of a low order of acute oral toxicity in rats (Levinskas et al., 1966). Acute oral/toxicity data for various species are reported as follows:

Species	Sex	Route	LD ₅₀ (mg/kg bwt)	Reference
Rat	Male Female	Oral	2700 4700	Levinskas et al. (1966)
Guinea-pig	Female	Oral	450	Struyk et al. (1958)
Rabbit	Male	Oral	1400	Levinskas et al. (1966)

IX.B.4 Sub-chronic Toxicity Studies

Rat

Oral administration of natamycin at doses of 50-70 mg/kg bwt per day for 5-10 weeks had no effect on the growth, clinical chemistry or hematology parameters, or tissues of rats. A daily oral dose of 150 mg/kg bwt for 9 weeks caused some growth inhibition, and a daily dose of 500 mg/kg bwt caused 30% of the rats to die within 2 weeks (Struyk, 1958).

Groups of 20 male and 20 female rats were fed diets containing natamycin at a concentration of 0, 125, 500, 2000, or 8000 mg/kg for 94-96 days. None of the five deaths observed could be attributed to treatment. Growth was retarded and food intake was diminished at the two highest concentrations. The results of hematological examinations and organ weights were within normal limits, and no gross or microscopic lesions were found that could be attributed to natamycin (Levinskas et al., 1966).

IX.B.5 Chronic Toxicity and Carcinogenicity studies

Chronic Toxicity and Carcinogenicity in Rats

Groups of 35-40 male and female rats received diets containing natamycin at a concentration of 0, 125, 250, 500, or 1000 ppm (mg/kg diet) for 2 years. The animals remained in good health, and their survival was unaffected by treatment. Inhibition of growth rate and diminished food intake were seen only for animals of each sex receiving the highest concentration. The results of hematological investigations and determination of organ weights and gross and microscopic lesions showed no differences between treated and control groups. The numbers and types of tumors found in natamycin-treated rats were not significantly different from those in untreated animals (Levinskas et al., 1966). The NOAEL for chronic oral dosing in rats is considered to be 500 ppm equivalent to a daily intake of 25 mg/kg bwt.

Chronic Toxicity in Dogs

Groups of three male and three female beagle dogs received diets containing natamycin at a concentration of 0, 125, 250, or 500 ppm (mg/kg diet) for 2 years. All but one dog that received 250 ppm bwt survived for 2 years; the death was considered unrelated to exposure to natamycin. No effect was seen on food intake, but males receiving the highest concentration did not grow as rapidly as controls initially, and after 15 months, when the dietary intake was reduced, some animals were unable to maintain a satisfactory body weight. The results of hematological and clinical chemical studies revealed no abnormalities. No effects of

significance were found on organ weights, and gross and microscopic examination showed no pathological changes (Levinskas et al., 1966). Based on the findings of reduced body weight in the 500 ppm group, the NOAEL was set at 250 ppm equivalent to 6 mg/kg bwt/day.

IX.B.6 Observations in Humans after Oral Treatment

A drug study (Newcomer, 1960) was performed to determine the effectiveness of natamycin by the oral or intra muscular route which would be of considerable advantage in the treatment of the systemic mycoses. A group of 10 patients with systemic mycoses received oral doses of 50-1000 mg/day for 13-180 days. Nausea, vomiting, and diarrhea occurred in those receiving 600-1000 mg/day and limited the use of the drug in the adults. Newcomer concluded that natamycin did not offer promise as an effective oral anti-fungal agent for the treatment of systemic mycoses. Raab (1973) also confirms in cases of systemic mycoses the absence of any beneficial effect.

In the latest EFSA opinion (EFSA, 2009), it was mentioned that the antifungal properties of natamycin were originally used in the development of products for the treatment of topical fungal disorders. The only significant remaining human therapeutic use for natamycin is in the treatment of fungal keratitis.

IX.C Conclusion - Literature Studies

In experimental animals, natamycin shows a low order of toxicity upon oral ingestion (Struyk et al., 1958; Levinskas et al., 1966). Raab (1973) stated that natamycin was not absorbed from the gut in animals or humans and this would explain the apparent lack of systemic toxicity via the oral exposure route.

The adverse effects observed in oral toxicity studies in experimental animals consisted of a decrease in food intake with a subsequent decrease in the rate of body weight gain, and gastrointestinal irritation and diarrhea, with dogs being the most sensitive species to these effects (Levinskas et al., 1966). These gastro-intestinal effects may result from effects of natamycin on the fungal gut flora, which could lead to impaired food digestion and availability of (micro-) nutrients.

The levels causing no toxicological effects in chronic oral toxicity studies in rats and dogs were 25 and 6 mg/kg bwt/day, respectively (Levinskas et al., 1966). In man mild gastrointestinal symptoms begin to appear at daily dosage levels of about 5 mg/kg bwt (Newcomer, 1960), although much higher dosage levels have been taken without ill-effects being observed. The level causing no adverse effects in man was estimated to be 200 mg/per day, equivalent to 3 mg/kg bwt/day (Newcomer, 1960).

Given that the level of dietary consumption for the proposed use of natamycin in yogurt and cheese as discussed in Section VIII.B is well below the ADI of 0.3 mg/kg bwt/day, there would appear to be an adequate safety margin for long term, daily consumption of yogurt containing natamycin.

Neither natamycin nor its degradation products were found to be mutagenic in bacterial reverse mutation assays (Khoudokormoff, 1977 and 1983).

The current Acceptable Daily Intake of 0.3 mg/kg bodyweight is based on observations of gastrointestinal effects in humans, using an uncertainty factor equal to 10 (given that this dose was derived from human data). The proposed application of natamycin in yogurt leads to an oral route of human exposure, in addition to the currently approved (grated/shredded) cheese application in the US.

In view of the high margin of safety provided by the results of chronic oral toxicity studies with natamycin in rats and dogs, the long history of safe use in grated/shredded cheese, and human data illustrating lack of significant absorption from the digestive tract, more than adequate toxicity data exists to support the safe use of natamycin in yogurt.

IX.D Toxicological studies

To confirm further the safety of natamycin, the following toxicological studies were conducted by or commissioned by DSM. Several of these DSM studies were submitted to the WHO/JECFA for their safety evaluation in 2002, which is clearly indicated in the reference list for each study.

IX.D.1 Absorption, Distribution and Excretion

Rat

The distribution of natamycin was studied by autoradiographic and bioautographic techniques (Blankwater & Hesper, 1979). In the autoradiographic study, five female Wistar rats were each given a single dose of 50 mg/kg bwt of [¹⁴C]natamycin (50 mg in 5 ml of 1% amylum) orally. In the bioautographic study, four female rats were each given a single dose of 50 mg/kg bwt (70 mg in 7 ml of 1% amylum) orally. In the bioautographic study, the antibiotic activity of the sections was evaluated by exposure on Whiffen agar plates inoculated with *Saccharomyces cerevisiae* strain ATCC 9763.

In the autoradiographic study, radiolabel was confined to the gastrointestinal tract after 93 days' exposure (1 h, esophagus, stomach, small intestine; 2 h, esophagus, stomach, small intestine, caecum; 4 h, stomach, small intestine, caecum, colon; 8 h, stomach, intestine; 24 h, caecum, colon). After 150 days' exposure, radiolabel was visible only faintly after magnification of the pictures, in the liver, kidneys, and fatty tissue, in addition to the gastrointestinal tract. In the bioautographic study, the antibiotic activity of natamycin was restricted to the gastrointestinal tract (1 h, stomach, small intestine; 4 h, stomach, small intestine, caecum; 8 h, stomach, small intestine, caecum) and lasted less than 24 h. No antibiotic activity was observed in the colon. The results of the autoradiographic study indicate that natamycin is minimally absorbed into the bloodstream and excreted almost entirely in the feces. The lack of antibiotic activity and the presence of radiolabel in the caecum and colon 24 h after dosing are consistent with the breakdown of natamycin into microbiologically inactive compounds by bacterial flora in the caecum and colon. This study demonstrates that natamycin is not absorbed to a significant extent, is inactivated in the caecum and colon by bacteria, and lacks antibiotic activity.

Human

No natamycin (< 1 µg/mL) could be detected in the blood after ingestion of 500 mg by human subjects (Anonymous, 1968).

IX.D.2 Mutagenicity Studies

Chromosome aberration test

The clastogenicity of natamycin was tested in a Chromosome aberration test in cultured human lymphocytes (OECD 475). Both in the presence and absence of a metabolic system, natamycin did not induce chromosomal aberrations, leading to the conclusion that natamycin is not clastogenic under the conditions employed in the test (Meerts, 2002).

IX.D.3 Acute Toxicity Studies

Acute Oral Toxicity

The test substance, Natamycin Instant, was evaluated for its acute oral toxicity potential in female albino rats when administered as a gavage dose at a level of 5000 mg/kg. Since the test substance failed the limit test the main test was conducted following the up-and-down procedure (WIP) at 175, 550, 1750 and 5000 mg/kg. The study was terminated following the stopping rules of this procedure. Mortality occurred only at the 5000 mg/kg level. Clinical signs included activity decrease, ataxia, diarrhea, piloerection, polyuria, stained muzzle/tail and sensitivity to sound; animals exhibited signs on Days 1-8. There was no effect on body weight gain in animals surviving to termination. Abnormal necropsy findings occurred only in the animals dying on test, and pertained to facial and body areas, liver and contents of the gastrointestinal tract. The acute oral LD₅₀ was estimated to be 5000 mg/kg (Kuhn, 2009).

Acute Dermal Toxicity in Rats

The dermal toxicity potential and relative skin irritancy was evaluated by applying a single dose of 5050 mg/kg natamycin moistened with 1.0 ml deionized water/g test substance, to the intact skin of albino rats. No mortality, no clinical signs of toxicity or signs of dermal irritation were observed at any time throughout the study. There was no effect on body weight gain, with the exception of one animal that failed to gain weight during the first week. The gross necropsy conducted at termination of the study revealed no observable abnormalities. The estimated LD₅₀, as indicated by the data, was determined to be greater than 5050 mg/kg (Kuhn, 2008a).

Acute Inhalation Toxicity in Rats

The acute inhalation toxicity potential of natamycin was evaluated in male and female albino rats by applying four hours exposure to an aerosol generated from the undiluted test substance (fine powder) at a level of 2.39 mg/L. There was no mortality during the study. Clinical signs included activity decrease and piloerection, which were no longer evident by Day 3. Body weights were unaffected by exposure. The gross necropsy revealed no observable abnormalities. As indicated by the data, the acute inhalation LC50 is greater than 2.39 mg/L (Crutchfield, 2008).

IX.D.4 Sub-chronic Toxicity Studies

Dog

Groups of two male and two female beagle dogs were given diets containing natamycin at a target concentration of 0, 375, or 750 mg/kg (equivalent to 0, 12, and 25 mg/kg bwt per day) for 3 months. The natamycin was obtained in micronized form and was 90.5% pure. The animals were monitored for clinical changes, body weight, food consumption, hematological, clinical chemical and urinary alterations, electrocardiography (wave intervals and heart rate at weeks 0, 4, 8, and 12), ophthalmology, and pupillary reactions. After being killed by an intravenous overdose of pentobarbital, all animals were necropsied, and the weights of the thymus, heart, liver, kidneys, adrenals, spleen, and testes were measured and gross lesions noted. The tissues preserved in buffered formaldehyde saline and examined microscopically were brain, thyroid, thymus, lung, heart, liver, kidneys, adrenals, spleen, pancreas, lymph nodes, urinary bladder, ovaries, testes, stomach, ileum, colon, jejunum, caecum, and esophagus. The statistical evaluations included analysis of variance and the Student t test.

No dose- or treatment-related effects were seen in males or females with respect to mortality rate, food consumption, body weight, hematological, clinical chemistry, or urinary end-points, electrocardiography, ophthalmology, absolute and relative organ weights, gross pathology, and histopathology. Apart from diarrhea, more frequently seen in the 750 mg/kg/day dosage group than in the 375 mg/kg/day and control groups, no indication of systemic and dose-related toxicity were seen. From these data, it may be concluded that dietary administration of the test-batch for 90-days produces no toxic manifestations and that the presence of diarrhea must be seen as a local effect. Due to this difference between the local

irritation and systemic toxicity further investigation with higher dosages is not easily possible. It can be concluded that the systemic toxicity lies considerably higher than 750 ppm (approx. 25 mg/kg bwt/day), (van Eeken, 1984).

These sub-chronic studies reveal that the toxic levels of natamycin are very much higher than the expected dietary consumption rates from the proposed use in yogurt, suggesting the proposed use does not introduce any new safety concerns with regard to subchronic exposures to natamycin in yogurt.

IX.D.5 Developmental Toxicity studies **Reproductive/Multigeneration study in rats**

Groups of 10 male and 20 female rats were given a diet containing natamycin providing a dose of 0 (two groups), 5, 15, 50, or 100 mg/kg bwt per day for 11 weeks. These formed the F0 generation of a three-generation study of reproductive toxicity, two litters being produced in each generation. Animals at 100 mg/kg bwt had an increased number of fetuses born dead, a decrease in the number born alive, and a decrease in the number surviving at 21 days. The weight of pups was depressed in the second litters of the F0 and F1 generations and both litters of the F2 generation. However, the fertility, gestation, viability, and lactation indices were within normal limits for both litters of all three generations. The doses of 5, 15, and 50 mg/kg bwt had no detectable effect on growth or reproduction (Cox et al., 1973). The NOAEL for reproductive toxicity is therefore considered to be 50 mg/kg bwt.

Developmental Toxicity study in rats

Groups of 20 female rats from the second litters of the F1 generation of the three-generation study of reproductive toxicity were reared to maturity on control diet and mated with untreated males. The females were given the same dose of natamycin as their parents (0, 5, 15, 50, or 100 mg/kg bwt per day) by gastric intubation during the 6-15 days of gestation and were killed and examined on day 20. No differences were found between control and test animals in respect of the numbers of pregnancies, live litters, implantation sites, resorption sites, live and dead fetuses, or skeletal and soft tissue abnormalities (Cox et al., 1973). The NOAEL for developmental toxicity in rats is therefore considered to be at least 100 mg/kg bwt, which is well above the levels of exposure expected from the proposed levels of use.

Developmental Toxicity study in rabbits

Groups of 10-12 female rabbits were given natamycin at a dose of 0, 5, 15, or 50 mg/kg bwt per day by gavage on days 6-18 of gestation. They were examined on day 29, and the numbers of corpora lutea, implantation sites, resorption sites, and live and dead fetuses were recorded. No adverse effects on nidation or maternal or fetal survival were found. The number of abnormalities seen in the soft or skeletal tissues did not differ from that occurring spontaneously in controls (Bailey & Morgareidge, 1974). The NOAEL for developmental toxicity in rabbits is therefore considered at least 50 mg/kg bwt.

IX.D.6 Studies on Degradation Products

The acute intraperitoneal toxicity of natamycin and three potential metabolites was determined in mice (van Eeken, 1976). The three decomposition products were aponatamycin, mycosamine and dinatamycinolidediole and were selected because they can be formed by gastrointestinal degradation as well as on storage. The natamycin was administered as a 1% amyllum suspension and the metabolites as a solution in physiological saline. A gender difference in toxicity was found for natamycin but not for the metabolites.

The following LD₅₀ values were calculated:

	LD₅₀ (mg/kg bwt)	Range (mg/kg bwt)
Natamycin	950	759-1240
Natamycin males	1636	1281-2089
Natamycin females	421	190-937
Aponatamycin	3189	2651-3837
Mycosamine HCL	3722	3218- 4304
Dinatamycinolidediole	> 4000	

It can be concluded that the acute toxicity of the breakdown products of natamycin is much lower than of natamycin itself.

IX.D.7 Irritation and Sensitization Studies

Acute eye irritation study in rabbits

The acute eye irritation potential of natamycin was tested on albino rabbits by applying 43.7 mg natamycin (0.1 mL by volume) into the conjunctival sac of the right eye of each animal. All treated eyes were washed with room temperature deionized water for one minute immediately after recording the 24-hour observation.

A maximum average irritation score of 62.0, severely irritating, was obtained 1 hour after treatment, but there were no positive effects exhibited in any eyes at 24 hours after treatment. Hence, natamycin was assigned Toxicity Category IV (Criteria: minimal effects clearing in less than 24 hours. No “positive” effect at 24 hours) (Kuhn, 2008b).

Acute dermal irritation study in rabbits

The acute dermal irritation potential of natamycin was evaluated in albino rabbits by applying 500 mg of test substance moistened with 0.5 mL of deionized water and covered with a semi-permeable dressing on one test site per animal. The test substance was kept in contact with the skin for 4 hours. Observations for dermal irritation and defects were made at 1, 24, 48, and 72 hours after removal of the dressings. Based on the PII (Primary Irritation Index) of 0.1, the test substance is rated slightly irritating. Based on the scores at the 72-hour observation only, the test substance is assigned to Toxicity Category IV (Non-irritating, mild, or slight irritation at 72 hours) (Kuhn, 2008c).

Skin sensitization: local lymph node assay in mice

Skin sensitization was tested in female mice by applying appropriate dilutions of natamycin (25% or 50%) in propylene glycol vehicle, or undiluted test substance in a volume of 25 μ L to the dorsum of each ear once a day for 3 consecutive days. The test substance produced a stimulation index of <3 in all groups of test animals, and is therefore considered non-sensitizing (defined as producing a positive response) (Kuhn, 2008d).

IX.D.8 Allergenicity

No allergic sensitization occurred among 111 patients being treated with natamycin for a variety of conditions (Grupper, 1961). As noted in the WHO evaluation of natamycin (JECFA, 2002), no history of allergic reactions was found in 73 workers engaged for an average of 5 years in the manufacture of natamycin, and no allergic reactions were found in the 71 who were tested with cutaneous or intradermal challenge doses (Malten, 1967). Repeated patch tests on 102 patients with various forms of eczema failed to demonstrate any sensitizing potential of natamycin (Malten, 1968).

Evidence from these studies suggests that natamycin is not likely to be an allergen.

DSM has concluded that the data that it has and the public data and information allow it to conclude that there is no published or unpublished data that suggest there is an allergen causing protein from the fermentation media in the finished natamycin product. To reach that conclusion, DSM relies on:

1. The Enzyme Technical Association in 2004 conducted a survey of its members, and collected information on the possible presence of protein from the fermentation media in the final enzyme products, which are made in a similar fashion to natamycin, through fermentation and purification. ETA provided the supporting data and information to FDA in a letter in 2005, and sent an accompanying public statement which is posted on ETA's website. The statement concludes that no allergens protein from the fermentation medium has been found in finished enzyme products, and states that regulatory bodies in both the EU and Japan have concluded that enzyme preparations do not pose an allergen risk that would require allergen labelling on the final product. Further, ETA points out that the typical manufacturing process of enzyme preparations includes a step to separate the biomass and fermentation media from the enzyme, as does the natamycin manufacturing process. This step ensures the product purity and stability, and would likely remove most proteins present in the fermentation media. A copy of the public statement from the ETA website is attached as Annex IX-2.

2. In addition, the Food Allergy Research and Resource Program (FARRP) issued a paper in August of 2013 which concluded that any *de minimis* amount of fermentation media protein that survived the fermentation

process will not cause a significant public health risk to the consumer. FARRP also underscores the fact that the proteins would likely be removed during the filtration of the enzyme product, as discussed by ETA. Further, FARRP indicates that there is no reliable assay that could be used to detect the presence of most allergen proteins in the final enzyme products, as the proteins would likely be degraded fragments that would not reach levels of quantization available with current commercial ELISA assays. The full August 2013 statement, provided as Annex IX-3, clearly concludes that that any protein allergen present in the final enzyme product would not be present at a level that requires labelling or present at a level that raises a public health concern. Again, because the manufacturing process for enzymes and natamycin production are similar, DSM has no reason to believe this conclusion would not apply to natamycin.

3. In addition, DSM has data from a study where wheat derived carbohydrates were used during fermentation, and an analysis after the fermentation shows the absence of gluten despite a detection limit of 10 parts per million (ppm). Finally, a soy flour that was used as a fermentation media, and post-fermentation analysis of the enzyme product revealed that no soy residue was present, with a level of detection of 0.5 ppm.

Finally, it is our understanding that a search of the scientific literature will not result in a reported allergic reaction from an enzyme caused by the fermentation medium. The ETA has conducted similar literature searches in the past, with no findings of allergic reactions due to fermentation media. The fermentation media as noted above is consumed in the process, and is removed with subsequent purification and filtration steps used in the enzyme production process. There is no evidence to support that a level of protein from the fermentation media exists in the final product which would cause an allergic reaction.

X Conclusion of the Dossier

On the basis of the results of toxicity studies, it can be concluded that natamycin can safely be ingested for long periods of time at the proposed levels of use. The toxicity and mutagenicity studies demonstrated the safety of natamycin, and showed no systemic toxicity or mutagenicity across a variety of test conditions. The current ADI is based mainly on local effects on the gut flora and was derived in a conservative fashion with a high safety margin.

Application and consumer intake data indicate a cumulative estimated 2-day average daily intake for the total US population at the 90th percentile of 0.023 mg/kg-bw/day. This includes consumption of cheese as well as yogurt. This value is well below the current ADI of 0.3 mg/kg-bw/day.

The rationale for the dosage of 5 ppm natamycin in yogurt is to provide sufficient preservative action till the end of shelf life which is dependent of the conditions of the various yogurt products as made throughout the dairy products industry. Based upon these factors, it is DSM's conclusion that natamycin is GRAS for its intended use in yogurt at a maximum dosage level of 5 ppm.

ANNEXES

- II.C-1 Overview natamycin specifications in Europe, CODEX/JECFA, USA FDA & FCC
- VII-1 Global legislations on natamycin as Food Additive in major countries
- VIII-1 Exponent, Inc., (2013) Estimated daily intake of natamycin from proposed uses in yogurt products, Report 1304507.000-2824
- IX-1 JECFA, 2002 Natamycin, Safety Evaluation of certain food additives and contaminants,
Authors: Dr A. Mattia, Dr C. Cerniglia, J. Baines,
Report of the Joint FAO/WHO Expert Committee on Food Additives (57th meeting), WHO Food Additives Series 48, pp 49 - 76
- IX-2 ETA position paper on food allergen labeling of microbially derived enzymes under FALCPA as it applies to fermentation media raw materials
- IX-3 Expert opinion statement Food Allergy Research & Resource Program, University of Nebraska, Testing of microbially derived enzymes for potential allergens from fermentation media raw materials



Annex II.C-1

Overview Natamycin specifications in Europe, CODEX/JECFA, USA FDA & FCC

Overview Natamycin specifications in Europe, CODEX/JECFA, USA FDA & FCC				
Parameter	EU Regulation 231/2012	CODEX/JECFA	FDA 21 CFR 172.155	Food Chemical Codex 8th ed.
Code	E 235	INS no 235	-	INS 235
Synonym	Pimaricin	Pimaricin	Pimaricin	Pimaricin
Definition	Natamycin is a fungicide of the polyene macrolide group, and is produced by natural strains of <i>Streptomyces natalensis</i> or of <i>Streptomyces lactis</i>	A fungicidal antimycotic of the polyene macrolide group. It is produced by several species of <i>Streptomyces</i> . The commercial product may contain up to three moles of water.	A polyene macrolide, antimycotic substance	Antimycotic
CAS		7681-93-8	7681-93-8	7681-93-8
Einecs	231-683-5			
Chemical formula	C ₃₃ H ₄₇ O ₁₃ N	C ₃₃ H ₄₇ NO ₁₃	C ₃₃ H ₄₇ NO ₁₃	C ₃₃ H ₄₇ NO ₁₃
Molecular weight	665.74	665.74	665.7	665.73
Assay	Content not less than 95% on the anhydrous basis	Not less than 95.0% calculated on the dried basis. Method of assay: High Performance Liquid Chromatography	97 percent ± 2 percent on an anhydrous basis.	Acceptance criteria: not less than 97.0 % and not more than 102.0 % C ₃₃ H ₄₇ NO ₁₃ , calculated on the anhydrous basis.
Description	White to creamy-white crystalline powder	White to creamy-white, almost odourless, crystalline powder	a polyene macrolide antimycotic substance	Natamycin occurs as an off-white to cream colored powder that may contain up to 3 moles of water. It melts with decomposition at about 280°. It is practically insoluble in water, slightly soluble in methanol, and soluble in glacial acetic acid and in dimethylformamide.

Overview Natamycin specifications in Europe, CODEX/JECFA, USA FDA & FCC				
Parameter	EU Regulation 231/2012	CODEX/JECFA	FDA 21 CFR 172.155	Food Chemical Codex 8 th ed.
Identification				
A. Color reactions	<p>On adding a few crystals of natamycin on a spot plate, to a drop of:</p> <ul style="list-style-type: none"> Concentrated hydrochloric acid, a blue color develops; Concentrated phosphoric acid, a green color develops; which changes into pale red after a few minutes. 	<p>On adding a few crystals of natamycin on a spot plate, to a drop of:</p> <ul style="list-style-type: none"> Concentrated hydrochloric acid, a blue color develops; Concentrated phosphoric acid, a green color develops which changes into pale-red after a few minutes. 		
B. Spectrometry	<p>A 0.0005% w/v solution in 1% methanolic acetic acid solution has absorption maxima at about 290 nm, 303 nm and 318 nm, a shoulder at about 280 nm and exhibits minima at about 250 nm, 295.5 nm and 311 nm.</p>	<p><u>Infrared absorption:</u> The infrared spectrum of a potassium bromide dispersion of the sample corresponds with the reference infrared spectrum in Appendix A.</p> <p><u>Ultraviolet absorption:</u> A solution of 5 mg/l of the sample in 0.1% glacial acetic acid in methanol has absorption maxima at about 290, 303 and 318 nm, a shoulder at about 280 nm and exhibits minima at about 250, 295.5 nm and 311 nm. See Appendix B.</p>		<p><u>Ultraviolet absorption:</u> The spectrum of the <i>Sample solution</i> exhibits maxima and minima at the same wavelengths as those in the spectrum of the <i>Standard solution</i>.</p>
C. pH	5.5 to 7.5 (1% w/v solution in previously neutralised mixture of 20 parts dimethylformamide and 80 parts of water).	5.0 – 7.5 (1.0% w/v suspension in demineralized water)		pH determination, Appendix IIB acceptance criteria: between 5.0 and 7.5
D. Specific rotation	$[\alpha]_D^{20} = +250^\circ$ to $+295^\circ$ (a 1% w/v solution in glacial acetic acid, at 20 °C and calculated with reference to the dried material)	$[\alpha]_D^{20} = +250^\circ$ to $+295^\circ$ (1% w/v solution in glacial acetic acid)		Appendix IIB. Acceptance criteria: $[\alpha]_D^{20}$ between $+276^\circ$ and $+280^\circ$ (10 mg/ml in glacial acetic acid)
Solubility		Practically insoluble in water, in lipid and in mineral oils; slightly soluble in methanol; soluble in glacial acetic acid and dimethylformamide.		

Overview Natamycin specifications in Europe, CODEX/JECFA, USA FDA & FCC				
Parameter	EU Regulation 231/2012	CODEX/JECFA	FDA 21 CFR 172.155	Food Chemical Codex 8 th ed.
Purity				
Water				Water determination Appendix IIB Acceptance criteria: between 6.0% and 9.0%
Loss on drying	Not more than 8% (over P ₂ O ₅ , in vacuum at 60 °C to constant weight)	Not more than 8.0% (60 °, over P ₂ O ₅ , pressure less than 5 mm Hg)		
Sulphated ash	Not more than 0.5%	Not more than 0.5% Test 2 g of the sample (Method I)		
Arsenic	Not more than 3 mg/kg		Not more than 1 part per million.	
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods".		Lead limit test, Flame Atomic Absorption Spectrophotometric Method, Appendix IIIb. Acceptance criteria: Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg			
Heavy metals (as Pb)	Not more than 10 mg/kg		Not more than 20 parts per million.	
Microbiological criteria:				
Total viable count	Not more than 100/g			



Annex VII-1

Global legislations on natamycin in major countries

Global legislations on natamycin in major countries

Country	Food legislation / Guidance
EU	EU Regulation (EC) No 1333/2008 Available at: http://eur-lex.europa.eu/Result.do?T1=V1&T2=2008&T3=1333&RechType=RECH_consolidated&Submit=Search
USA	US regulation 21CFR 172.155 Available at: http://www.gpo.gov/fdsys/pkg/CFR-2012-title21-vol3/pdf/CFR-2012-title21-vol3-sec172-155.pdf
USA	US Memorandum prepared by Milk Safety Team (HFS-626) for all Regional Food and Drug Directors in January 2007, pages 3 and 4: Available at: http://www.fda.gov/downloads/Food/FoodSafety/Product-SpecificInformation/MilkSafety/CodedMemoranda/MemorandaofInformation/UCM073760.pdf
Canada	Canada F&DR Division 16 Food Additives, Part III Food additives that may be used as Class III preservatives: Available at: http://laws-lois.justice.gc.ca/eng/regulations/C.R.C.%2C_c._870/page-158.html#docCont
Australia/ NZ	Australia&New Zealand Food Standards Code, Standard 1.3.1 Food Additives Schedule 1 Available at: http://www.comlaw.gov.au/Details/F2011C00892
China	China GB2760 Food Additives Available at: http://www.chinafoodsafety.net/newslist/newslist.jsp?anniu=Law_1
Japan	Japan Food Sanitation Law - Standards for use Available at: http://www.ffcr.or.jp/zaidan/FFCRHOME.nsf/7bd44c20b0dc562649256502001b65e9/8a4352b95978b195492569990007fbaa/\$FILE/Standards%20for%20Use%2011Dec.27.pdf
South Africa	South Africa Regulations - Preservatives and antioxidants, Published under Government notice No. R965 Available at: http://www.doh.gov.za/docs/regulations/1977/reg0965.pdf
CODEX /JECFA	CODEX/JECFA Monograph Natamycin Available at: http://www.codexalimentarius.net/gsfaonline/additives/details.html?id=208



Annex VIII-1

Exponent, Inc.
Center for chemical regulation and food safety

Estimated daily intake of natamycin from proposed uses in yogurt products

The logo for Exponent, featuring the word "Exponent" in a white serif font with a registered trademark symbol, set against a dark teal background. A large, faint, light teal version of the "Exponent" logo is visible in the background of the teal section.

Exponent®

Center for Chemical Regulation and Food Safety

**ESTIMATED DAILY INTAKE OF
NATAMYCIN FROM PROPOSED
USES IN YOGURT PRODUCTS**

**ESTIMATED DAILY INTAKE OF
NATAMYCIN FROM PROPOSED
USES IN YOGURT PRODUCTS**

Prepared for

Robert Zega
DSM Nutritional Products
45 Waterview Blvd.
Parsippany, NJ 07054

Prepared by

Exponent, Inc.
1150 Connecticut Avenue, NW
Suite 1100
Washington, DC 20036

February 18, 2014

© Exponent, Inc.

1304507.000 - 3956

i

Contents

	<u>Page</u>
List of Tables	iii
List of Acronyms	iv
Introduction	1
Data and Methods	2
Background Use	2
Proposed Use	2
Consumption Data	2
Analysis	3
Background EDI	4
Proposed Uses EDI	5
Cumulative EDI	5
Results	6
References	10
Appendix I. Food Code Included In Analysis	11

List of Tables

	<u>Page</u>
Table 1. Background estimated 2-day average daily intake of natamycin from approved uses in non-processed cheese by the total US population; NHANES 2007-10	6
Table 2. Estimated 2-day average daily intake of natamycin proposed for use in yogurt products by the total US population; NHANES 2007-10	7
Table 3. Cumulative estimated 2-day average daily intake of natamycin from background and proposed uses for the total US population and sub-populations (mg/day); NHANES 2007-10	8
Table 4. Cumulative estimated 2-day average daily intake of natamycin from background and proposed uses for the total US population and sub-populations (mg/kg-bw/day); NHANES 2007-10	9

List of Acronyms

CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
DHHS	Department of Health and Human Services
EDI	Estimated Daily Intake
FNDDS	Food and Nutrient Database for Dietary Studies
NCHS	National Center for Health Statistics
NHANES	National Health and Nutrition Examination Survey
USDA	U.S. Department of Agriculture
WWEIA	What We Eat in America

Introduction

At the request of DSM Food Specialties (DSM), Exponent, Inc. (Exponent) conducted an intake assessment to estimate the total daily intake of natamycin from proposed use in yogurt products by the overall US population. The background intake of natamycin was also assessed based on Code of Federal Regulation (CFR) approved uses of natamycin in finished cheese products to estimate cumulative intake from background and proposed new uses combined. The estimated daily intake (EDI) of natamycin is based on foods reported consumed in the What We Eat in America (WWEIA) dietary component of the National Health and Nutrition Examination Surveys (NHANES) 2007-2010. The sections below summarize the data, methods, and results.

Data and Methods

Background Use

According to the Code of Federal Regulations (CFR), natamycin is approved for use as an antimycotic in cheese in amounts not to exceed 20 ppm in the finished product (21CFR§172.155). Based on known uses of natamycin to inhibit mold spoilage in finished cheese products, Exponent included only non-processed cheese in the assessment.

Proposed Use

Natamycin is proposed for use in yogurt products at a minimum and maximum use level of natamycin of 5 ppm and 7 ppm, respectively. Examples of yogurt products include plain, flavored, fruit variety, and soy yogurt.

Consumption Data

Estimated food intakes intended for natamycin from background and proposed uses were based on food consumption records collected in the WWEIA component of NHANES conducted in 2007-2008 and 2009-2010 (NHANES 2007-2010). This continuous survey uses a complex multistage probability sample designed to be representative of the civilian U.S. population (NCHS 2010, 2012). The NHANES datasets provide nationally representative nutrition and health data and prevalence estimates for nutrition and health status measures in the United States. Statistical weights are provided by the National Center for Health Statistics (NCHS) to adjust for the differential probabilities of selection.

As part of the examination, trained dietary interviewers collected detailed information on all foods and beverages consumed by respondents in the previous 24 hour time period (midnight to midnight). A second dietary recall was administered by telephone three to ten days after the first dietary interview, but not on the same day of the week as the first interview. The dietary component of the survey is conducted as a partnership between the U.S. Department of

1304507.000 - 3956

2

component of the survey is conducted as a partnership between the U.S. Department of Agriculture (USDA) and the U.S. Department of Health and Human Services (DHHS). DHHS is responsible for the sample design and data collection, and USDA is responsible for the survey's dietary data collection methodology, maintenance of the databases used to code and process the data, and data review and processing. A total of 16,244 individuals in the survey period 2007-2010 provided 2 complete days of dietary recalls.

Consumption data in the NHANES survey are reported on an "as consumed basis". That is, if a survey participant consumed an apple pie, the consumption amount reported in the survey for that subject would be for the amount of pie consumed, and not for the ingredients (flour, butter, apples, sugar, etc.) used to make that pie. Exponent identified non-processed cheese and yogurt product foods reported consumed for background and proposed use of natamycin, respectively. The list of NHANES codes (and their description) that were captured in determining the foods with natamycin from background and proposed uses is provided in Appendix I. In cases where the component of the food is either intended for the background or proposed use of natamycin, Exponent utilized USDA's Food and Nutrient Database for Dietary Studies (FNDDS), version 5.0 (USDA, 2012), that translates the food as consumed into its corresponding ingredients (and gram amounts) or recipes. Consumption of the foods with background or proposed use of natamycin was estimated using the USDA recipes when the target food was a component of the reported food (e.g., yogurt component in gyro sandwich, non-processed cheese component in a burrito). Thus, for foods that contained the background or proposed use food (i.e., as an ingredient), only the target food component was captured in the analysis.

Analysis

Using the NHANES consumption data, Exponent estimated the daily intake of foods with approved and proposed uses of natamycin on a *per user* basis. In this analysis, a user is anyone who reported consuming any of the background and/or proposed foods on either of the survey days (USDA's user definition), as appropriate. We identify each participant who reported

consuming the foods of interest on either of the survey days, and we use that individual's responses for both survey days. Zero consumption days are included in calculating that individual's average daily intake. For example, if someone reported consuming 240 grams of yogurt on day 1 and 120 grams of yogurt on day 2, his/her 2-day average yogurt consumption would be 180 grams $((240+120)/2)$. The analysis was limited to individuals who provided two complete and reliable dietary recalls as determined by NCHS. The 2-day average intakes by each individual were estimated using Exponent's Foods and Residues Evaluation Program (FARE[®] version 10.05) software. Exponent uses the statistically weighted values from the survey in its analyses. The statistical weights compensate for variable probabilities of selection, adjust for non-response, and provide intake estimates that are representative of the U.S. population.

Generally, the 2-day average intake of natamycin was estimated by multiplying the reported intake of foods from the 24-hr recall with the natamycin use level and the cumulative sum over the two 24-hr recalls was divided by two. Estimates were also derived on a bodyweight basis based on each participant's reported bodyweight.

Using the yogurt example mentioned above, an individual's 2-day average intake of natamycin from proposed use in yogurt at the maximum level of 7 ppm is derived as follows:

$$2 - \text{day average intake of natamycin} = \frac{\left(360 \text{ g yogurt} \times 7 \frac{\text{mg natamycin}}{\text{kg yogurt}} \times \frac{1 \text{ kg}}{1000 \text{ g}} \right)}{2 \text{ days}}$$

$$2 - \text{day average intake of natamycin} = 1.26 \text{ mg/day}$$

Background EDI

The estimated daily intake (EDI) of natamycin from background uses in non-processed cheese products were derived from food consumption data reported in the NHANES 2007-2010 in combination with the USDA FNDDS recipes database. As described above, for each participant with a complete 2-day dietary recall, intake of natamycin was derived by multiplying the sum of

the participant's reported intake of non-processed cheese on day 1 and day 2 of the survey with the maximum approved use level of 20 ppm (21CFR§172.155), then dividing by two.

Proposed Uses EDI

Similar to the background EDI of natamycin, estimates of natamycin from yogurt products were also derived from food consumption data reported in the NHANES 2007-2010 in combination with the USDA FNDDS recipes database. Exponent reviewed the list of food codes reported consumed during this survey period and identified yogurt products and foods containing yogurt as an ingredient. The two day average intake of natamycin from proposed uses in yogurt products were estimated for each individual by multiplying the sum of the participant's reported intake of the proposed use food (either reported eaten "as is" or as a portion of a food) on both days on day 1 and day 2 of the survey with the proposed use level, then dividing by two. Exponent derived two sets of natamycin estimates using the minimum and maximum use level of 5 ppm and 7 ppm, respectively.

Cumulative EDI

To estimate the 2-day average cumulative EDI for natamycin from background and proposed uses, each individual's 2-day average natamycin intake from background uses in non-processed cheese was added to their 2-day average natamycin intake from yogurt products reported consumed. Two sets of cumulative natamycin estimates were derived using the minimum and maximum use level of 5 ppm and 7 ppm, respectively, in proposed foods.

Results

The total estimated daily intake of natamycin from background and proposed uses by the overall US population in units of mg/day and mg/kg-bw/day are provided in Table 1 and 2 respectively. Daily cumulative natamycin intake estimates from combined background and proposed uses by the overall US population and children and adolescent sub-groups are provided in Table 3 (mg/day) and Table 4 (mg/kg-bw/day).

Table 1. Background estimated 2-day average daily intake of natamycin from approved uses in non-processed cheese by the total US population; NHANES 2007-10

Natamycin ¹	N-user ²	% Users	Per Capita		Per User	
			Mean	90th Percentile	Mean	90th Percentile
Total US Population						
mg/day	10,093	66	0.36	0.96	0.54	1.17
mg/kg-bw/day			0.006	0.016	0.009	0.020

¹ Based on maximum approved use level of natamycin at 20 ppm in non-processed cheese (21CFR§172.155).

² Un-weighted number of consumers; % user, per capita and per user estimates based on statistical weights provided by the National Center for Health Statistics (NCHS).

Table 2. Estimated 2-day average daily intake of natamycin proposed for use in yogurt products by the total US population; NHANES 2007-10

Natamycin	N-user ¹	% Users	Proposed Use Level at 5 ppm				Proposed Use Level at 7 ppm			
			Per Capita		Per User		Per Capita		Per User	
			Mean	90th Percentile	Mean	90th Percentile	Mean	90th Percentile	Mean	90th Percentile
Total US Population										
mg/day	2,067	14	0.07	0.31	0.51	0.92	0.10	0.43	0.71	1.29
mg/kg-bw/day			0.002	0.005	0.011	0.022	0.002	0.007	0.015	0.031

¹ Un-weighted number of consumers; % user, per capita and per user estimates derived using the statistical weights provided by the National Center for Health Statistics (NCHS)

Table 3. Cumulative estimated 2-day average daily intake of natamycin from background and proposed uses for the total US population and sub-populations (mg/day); NHANES 2007-10

Natamycin	N-user ¹	% Users	Proposed Use Level at 5 ppm				Proposed Use Level at 7 ppm			
			Per Capita		Per User		Per Capita		Per User	
			Mean	90th Percentile	Mean	90th Percentile	Mean	90th Percentile	Mean	90th Percentile
Total US Population	10,745	70.6	0.43	1.13	0.61	1.31	0.46	1.20	0.65	1.45
Adults 19+ y	6,517	70.1	0.45	1.18	0.64	1.38	0.48	1.29	0.68	1.52
Children 1-3 y	910	76.3	0.34	0.84	0.45	0.95	0.38	0.97	0.50	1.08
Children 4-6 y	681	75.0	0.36	0.90	0.49	1.08	0.40	1.04	0.54	1.18
Children 7-12 y	1,359	74.8	0.37	0.86	0.50	0.99	0.40	0.92	0.53	1.06
Males 13-19 y	684	73.9	0.52	1.28	0.70	1.46	0.53	1.33	0.72	1.60
Females 13-19 y	642	75.4	0.38	1.02	0.50	1.12	0.39	1.02	0.52	1.14

¹ Un-weighted number of consumers; % user, per capita and per user estimates based on statistical weights provided by the National Center for Health Statistics (NCHS)

Table 4. Cumulative estimated 2-day average daily intake of natamycin from background and proposed uses for the total US population and sub-populations (mg/kg-bw/day); NHANES 2007-10

Natamycin	N-user ¹	% Users	Proposed Use Level at 5 ppm				Proposed Use Level at 7 ppm			
			Per Capita		Per User		Per Capita		Per User	
			Mean	90th Percentile	Mean	90th Percentile	Mean	90th Percentile	Mean	90th Percentile
Total US Population	10,745	70.6	0.008	0.019	0.011	0.023	0.008	0.021	0.012	0.025
Adults 19+ y	6,517	70.1	0.006	0.015	0.008	0.018	0.006	0.016	0.009	0.020
Children 1-3 y	910	76.3	0.025	0.064	0.033	0.071	0.029	0.075	0.038	0.079
Children 4-6 y	681	75.0	0.018	0.047	0.024	0.051	0.020	0.053	0.026	0.059
Children 7-12 y	1,359	74.8	0.010	0.024	0.014	0.028	0.011	0.027	0.015	0.030
Males 13-19 y	684	73.9	0.008	0.019	0.011	0.023	0.008	0.019	0.011	0.023
Females 13-19 y	642	75.4	0.006	0.018	0.009	0.020	0.007	0.018	0.009	0.020

¹ Un-weighted number of consumers; % user, per capita and per user estimates based on statistical weights provided by the National Center for Health Statistics (NCHS)

References

Food and Drug Administration (FDA). 2000. 21CFR§172.155 Natamycin (pimaricin). US Government Printing Office. Available at: <http://www.gpo.gov/fdsys/pkg/CFR-2000-title21-vol3/pdf/CFR-2000-title21-vol3-sec172-155.pdf>.

National Center for Health Statistics (NCHS). 2012. National Health and Nutrition Examination Survey Data 2009-2010. Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Available via: http://www.n.cdc.gov/nchs/nhanes/search/nhanes09_10.aspx.

National Center for Health Statistics (NCHS). 2010. National Health and Nutrition Examination Survey Data 2007-2008. Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Available via: http://www.n.cdc.gov/nchs/nhanes/search/nhanes07_08.aspx.

US Department of Agriculture (USDA). 2012. USDA Food and Nutrient Database for Dietary Studies (FNDDS), 5.0. Beltsville, MD: US Department of Agriculture, Agricultural Research Service, Food Surveys Research Group. Available via: <http://www.ars.usda.gov/ba/bhnrc/fsrg>. Accessed 25 May 2013.

Appendix I. Food Code Included In Analysis

Foods included for background EDI analysis:

Food Code	Description
13210180	Pudding, Mexican bread (Capirotada)*
13252500	Barfi or Burfi, Indian dessert, made from milk and/or cream and/or Ricotta cheese*
14010000	Cheese, NFS*
14010100	Cheese, Cheddar or American type, NS as to natural or processed*
14100100	Cheese, natural, NFS
14101010	Cheese, Blue or Roquefort
14102010	Cheese, Brick
14103010	Cheese, Camembert
14103020	Cheese, Brie
14104010	Cheese, natural, Cheddar or American type
14104015	Cheese, natural, Cheddar or American type, reduced fat
14104020	Cheese, Cheddar or American type, dry, grated
14104200	Cheese, Colby
14104250	Cheese, Colby Jack
14104400	Cheese, Feta
14104600	Cheese, Fontina
14104700	Cheese, goat
14105010	Cheese, Gouda or Edam
14105200	Cheese, Gruyere
14106200	Cheese, Monterey
14106500	Cheese, Monterey, lowfat
14107010	Cheese, Mozzarella, NFS
14107020	Cheese, Mozzarella, whole milk
14107030	Cheese, Mozzarella, part skim
14107040	Cheese, Mozzarella, low sodium
14107060	Cheese, Mozzarella, nonfat or fat free
14107200	Cheese, Muenster
14107250	Cheese, Muenster, lowfat
14108010	Cheese, Parmesan, dry grated
14108015	Cheese, Parmesan, dry grated, reduced fat
14108020	Cheese, Parmesan, hard
14108060	Parmesan cheese topping, fat free
14108400	Cheese, Provolone
14108420	Cheese, provolone, reduced fat
14109010	Cheese, Swiss

Food Code	Description
14109020	Cheese, Swiss, low sodium
14109030	Cheese, Swiss, lowfat
14110010	Cheese, Cheddar or Colby, low sodium
14110030	Cheese, Cheddar or Colby, lowfat
14120010	Cheese, Mexican blend
14120020	Cheese, Mexican blend, reduced fat
14131000	Queso Anejo (aged Mexican cheese)
14131500	Queso Asadero
14132000	Queso Chihuahua
14133000	Queso Fresco
14201500	Cheese, Ricotta
14203510	Puerto Rican white cheese (queso del pais, blanco)
14610520	Cheese with nuts*
14620300	Topping from cheese pizza*
14620310	Topping from vegetable pizza*
14620320	Topping from meat pizza*
14630100	Cheese fondue*
14630200	Cheese souffle*
14630300	Welsh rarebit*
14650100	Cheese sauce
14650160	Alfredo sauce*
27111430	Chili con carne, NS as to beans, with cheese*
27111440	Chili con carne with beans and cheese*
27135110	Veal parmigiana*
27146200	Chicken or turkey with cheese sauce (mixture)*
27146250	Chicken or turkey cordon bleu*
27146300	Chicken or turkey parmigiana*
27150510	Scallops with cheese sauce (mixture)*
27212050	Beef and macaroni with cheese sauce (mixture)*
27213600	Beef and rice with cheese sauce (mixture)*
27220170	Sausage and rice with cheese sauce (mixture)*
27242310	Chicken or turkey and noodles with cheese sauce (mixture)*
27242350	Chicken or turkey tetrazzini*
27250110	Scallops and noodles with cheese sauce (mixture)*
27250130	Shrimp and noodles with cheese sauce (mixture)*
27311635	Beef, potatoes, and vegetables (including carrots, broccoli, and/or dark-green leafy), cheese sauce (mixture)*
27311640	Beef, potatoes, and vegetables (excluding carrots, broccoli, and dark-green leafy), cheese sauce (mixture)*
27313320	Beef, noodles, and vegetables (excluding carrots, broccoli, and dark-green leafy), (mushroom) soup (mixture)*
27315340	Beef, rice, and vegetables (excluding carrots, broccoli, and dark-green leafy), cheese sauce (mixture)*

Food Code	Description
27320120	Sausage, potatoes, and vegetables (including carrots, broccoli, and/or dark-green leafy), gravy (mixture)*
27320130	Sausage, potatoes, and vegetables (excluding carrots, broccoli, and dark-green leafy), gravy (mixture)*
27341050	Chicken or turkey, potatoes, and vegetables (excluding carrots, broccoli, and dark-green leafy), cheese sauce (mixture)*
27345440	Chicken or turkey, rice, and vegetables (including carrots, broccoli, and/or dark-green leafy), cheese sauce (mixture)*
27345450	Chicken or turkey, rice, and vegetables (excluding carrots, broccoli, and dark-green leafy), cheese sauce (mixture)*
27416300	Beef taco filling: beef, cheese, tomato, taco sauce*
27443150	Chicken or turkey divan*
27446315	Chicken or turkey garden salad with bacon (chicken and/or turkey, bacon, cheese, lettuce and/or greens, tomato and/or carrots, other vegetables), no dressing*
27446320	Chicken or turkey (breaded, fried) garden salad with bacon (chicken and/or turkey, bacon, cheese, lettuce and/or greens, tomato and/or carrots, other vegetables), no dressing*
27446360	Chicken or turkey caesar garden salad (chicken and/or turkey, lettuce, tomato, cheese), no dressing*
27446400	Chicken or turkey and vegetables (including carrots, broccoli, and/or dark-green leafy (no potatoes)), cheese sauce (mixture)*
27446410	Chicken or turkey and vegetables (excluding carrots, broccoli, and dark-green leafy (no potatoes)), cheese sauce (mixture)*
27460490	Julienne salad (meat, cheese, eggs, vegetables), no dressing*
27460510	Antipasto with ham, fish, cheese, vegetables*
27510700	Meatball and spaghetti sauce submarine sandwich*
27510950	Reuben sandwich (corned beef sandwich with sauerkraut and cheese), with spread*
27515050	Fajita-style beef sandwich with cheese, on pita bread, with lettuce and tomato*
27515070	Steak and cheese submarine sandwich, with fried peppers and onions, on roll*
27520410	Cuban sandwich, (Sandwich cubano), with spread*
27540200	Fajita-style chicken sandwich with cheese, on pita bread, with lettuce and tomato*
27540210	Wrap sandwich filled with chicken strips (breaded, fried), cheese, lettuce, and spread*
27540300	Wrap sandwich filled with chicken strips (broiled), cheese, lettuce, and spread*
27560330	Frankfurter or hot dog, with cheese, plain, on bun*
27560705	Sausage balls (made with biscuit mix and cheese)*
28110380	Salisbury steak with gravy, macaroni and cheese (frozen meal)*
28113110	Salisbury steak, baked, with tomato sauce, vegetable (diet frozen meal)*
28133110	Veal, breaded, with spaghetti, in tomato sauce (frozen meal)*
28140150	Chicken divan (frozen meal)*
28140730	Chicken patty, breaded, with tomato sauce and cheese, fettuccine alfredo,

Food Code	Description
	vegetable (frozen meal)*
28141050	Chicken patty parmigiana, breaded, with vegetable (diet frozen meal)*
28143080	Chicken with noodles and cheese sauce (diet frozen meal)*
28143150	Chicken and vegetable entree with noodles (diet frozen meal)*
28143170	Chicken in cream sauce with noodles and vegetable (frozen meal)*
28143220	Chicken in barbecue sauce, with rice, vegetable and dessert, reduced fat and sodium (diet frozen meal)*
28144100	Chicken and vegetable entree with noodles and cream sauce (frozen meal)*
28150210	Haddock with chopped spinach (diet frozen meal)*
28150220	Flounder with chopped broccoli (diet frozen meal)*
28315160	Italian Wedding Soup*
32105010	Egg omelet or scrambled egg, with cheese*
32105045	Egg omelet or scrambled egg, with cheese and dark-green vegetables*
32105055	Egg omelet or scrambled egg, with cheese and vegetables other than dark-green*
32105080	Egg omelet or scrambled egg, with cheese and ham or bacon*
32105081	Egg omelet or scrambled egg, with ham or bacon, cheese, and dark-green vegetables*
32105082	Egg omelet or scrambled egg, with ham or bacon, cheese, and vegetables other than dark-green*
32105085	Egg omelet or scrambled egg, with cheese, ham or bacon, and tomatoes*
32105119	Egg omelet or scrambled egg, with sausage, cheese, and vegetables other than dark-green*
32105121	Egg omelet or scrambled egg, with sausage and cheese*
32105126	Egg omelet or scrambled egg, with hot dog and cheese*
32105150	Egg omelet or scrambled egg, with cheese, beans, tomatoes, and chili sauce*
32105161	Egg omelet or scrambled egg, with chorizo and cheese*
32105190	Egg casserole with bread, cheese, milk and meat*
32400050	Egg white omelet or scrambled egg, with cheese*
41205020	Refried beans with cheese*
51154600	Roll, cheese*
51184030	Bread stick, soft, prepared with garlic and parmesan cheese*
53104300	Cake, carrot, diet*
53105500	Cake, chocolate, with icing, diet*
53440600	Strudel, cheese*
53440800	Strudel, cheese and fruit*
53452200	Pastry, Italian, with cheese*
53452450	Cheese pastry puffs*
53610200	Coffee cake, crumb or quick-bread type, cheese-filled*
54327950	Crackers, cylindrical, peanut-butter filled*
54328110	Cracker, sandwich-type, peanut butter filled, reduced fat*
54402500	Salty snacks, wheat- and corn-based chips*
54403050	Popcorn, flavored*

Food Code	Description
54408300	Pretzels, cheese-filled*
54420200	Multigrain mixture, bread sticks, sesame nuggets, pretzels, rye chips*
58100120	Burrito with beef, beans, and cheese*
58100130	Burrito with beef and cheese, no beans*
58100155	Burrito with beef, rice, and cheese*
58100160	Burrito with beef, beans, rice, and cheese*
58100220	Burrito with chicken, beans, and cheese*
58100230	Burrito with chicken and cheese*
58100245	Burrito with chicken, beans, cheese, and sour cream*
58100250	Burrito with chicken, rice, and cheese*
58100255	Burrito with chicken, beans, rice, and cheese*
58100320	Burrito with beans and cheese, meatless*
58100330	Burrito with rice, beans, cheese, sour cream, lettuce, tomato and guacamole, meatless*
58100350	Burrito with eggs and cheese, no beans*
58100360	Chilaquiles, tortilla casserole with salsa, cheese, and egg*
58100370	Chilaquiles, tortilla casserole with salsa and cheese, no egg*
58100520	Enchilada with beef, beans, and cheese*
58100530	Enchilada with beef and cheese, no beans*
58100620	Enchilada with chicken, beans, and cheese, tomato-based sauce*
58100630	Enchilada with chicken and cheese, no beans, tomato-based sauce*
58100720	Enchilada with beans and cheese, meatless*
58100800	Enchilada with cheese, meatless, no beans*
58101300	Taco or tostada with beef, cheese and lettuce*
58101320	Taco or tostada with beef, cheese, lettuce, tomato and salsa*
58101350	Soft taco with beef, cheese, lettuce, tomato and sour cream*
58101400	Soft taco with beef, cheese, and lettuce*
58101450	Soft taco with chicken, cheese, and lettuce*
58101460	Soft taco with chicken, cheese, lettuce, tomato and sour cream*
58101520	Taco or tostada with chicken, cheese, lettuce, tomato and salsa*
58101530	Soft taco with beef, cheese, lettuce, tomato and salsa*
58101600	Soft taco with bean, cheese, and lettuce*
58101610	Soft taco with bean, cheese, lettuce, and tomato and/or salsa*
58101615	Soft taco with bean, cheese, lettuce, tomato and/or salsa, and sour cream*
58101720	Taco or tostada with beans and cheese, meatless, with lettuce, tomato and salsa*
58101730	Taco or tostada with beans, cheese, meat, lettuce, tomato and salsa*
58101820	Mexican casserole made with ground beef, beans, tomato sauce, cheese, taco seasonings, and corn chips*
58101830	Mexican casserole made with ground beef, tomato sauce, cheese, taco seasonings, and corn chips*
58101910	Taco or tostada salad with beef and cheese, corn chips*
58101930	Taco or tostada salad with beef, beans and cheese, fried flour tortilla*

Food Code	Description
58101940	Taco or tostada salad, meatless, with cheese, fried flour tortilla*
58104080	Nachos with beef, beans, cheese, and sour cream*
58104090	Nachos with cheese and sour cream*
58104120	Nachos with beans and cheese*
58104130	Nachos with beef, beans, and cheese*
58104140	Nachos with beef and cheese*
58104180	Nachos with beef, beans, cheese, tomatoes, sour cream and onions*
58104250	Nachos with chicken or turkey and cheese*
58104260	Chalupa with beans, cheese, lettuce and tomato*
58104280	Chalupa with beef, cheese, lettuce, tomato and sour cream*
58104290	Chalupa with beef, cheese, lettuce, tomato and salsa*
58104320	Chalupa with chicken, cheese, lettuce, tomato and sour cream*
58104340	Chalupa with chicken, cheese, lettuce, tomato and salsa*
58104510	Chimichanga with beef, cheese, lettuce and tomato*
58104520	Chimichanga with beans and cheese, meatless, with lettuce and tomato*
58104530	Chimichanga with chicken and cheese*
58104710	Quesadilla with cheese, meatless*
58104730	Quesadilla with meat and cheese*
58105100	Pupusa, cheese-filled*
58105110	Pupusa, meat-filled*
58106200	Pizza, cheese, prepared from frozen, thin crust*
58106205	Pizza, cheese, prepared from frozen, thick crust*
58106210	Pizza, cheese, NS as to type of crust*
58106220	Pizza, cheese, thin crust*
58106225	Pizza, cheese, regular crust*
58106230	Pizza, cheese, thick crust*
58106240	Pizza, extra cheese, NS as to type of crust*
58106250	Pizza, extra cheese, thin crust*
58106255	Pizza, extra cheese, regular crust*
58106260	Pizza, extra cheese, thick crust*
58106300	Pizza, cheese, with vegetables, prepared from frozen, thin crust*
58106305	Pizza, cheese with vegetables, prepared from frozen, thick crust*
58106310	Pizza, cheese, with vegetables, NS as to type of crust*
58106320	Pizza, cheese, with vegetables, thin crust*
58106325	Pizza, cheese, with vegetables, regular crust*
58106330	Pizza, cheese, with vegetables, thick crust*
58106345	Pizza with cheese and extra vegetables, thin crust*
58106347	Pizza with cheese and extra vegetables, regular crust*
58106350	Pizza with cheese and extra vegetables, thick crust*
58106358	Pizza, cheese, with fruit, thin crust*
58106359	Pizza, cheese, with fruit, regular crust*
58106360	Pizza, cheese, with fruit, thick crust*

Food Code	Description
58106411	Pizza with chicken, thin crust*
58106412	Pizza with chicken, regular crust*
58106413	Pizza with chicken, thick crust*
58106441	Pizza with chicken and vegetables, thin crust*
58106442	Pizza with chicken and vegetables, regular crust*
58106443	Pizza with chicken and vegetables, thick crust*
58106462	Pizza with chicken and fruit, regular crust*
58106500	Pizza with meat, prepared from frozen, thin crust*
58106505	Pizza with meat, prepared from frozen, thick crust*
58106610	Pizza with meat other than pepperoni, NS as to type of crust*
58106620	Pizza with meat other than pepperoni, thin crust*
58106625	Pizza with meat other than pepperoni, regular crust*
58106630	Pizza with meat other than pepperoni, thick crust*
58106640	Pizza with extra meat, NS as to type of crust*
58106650	Pizza with extra meat, thin crust*
58106655	Pizza with extra meat, regular crust*
58106660	Pizza with extra meat, thick crust*
58106710	Pizza with meat and vegetables, NS as to type of crust*
58106720	Pizza with meat and vegetables, thin crust*
58106730	Pizza with meat and vegetables, thick crust*
58106733	Pizza with extra meat and extra vegetables, prepared from frozen, thin crust*
58106734	Pizza with extra meat and extra vegetables, prepared from frozen, thick crust*
58106735	Pizza with extra meat and extra vegetables, NS as to type of crust*
58106736	Pizza with extra meat and extra vegetables, thin crust*
58106737	Pizza with extra meat and extra vegetables, thick crust*
58106738	Pizza with extra meat and extra vegetables, regular crust*
58106750	Pizza with meat and fruit, thin crust*
58106755	Pizza with meat and fruit, regular crust*
58106760	Pizza with meat and fruit, thick crust*
58106820	Pizza with beans and vegetables, thin crust*
58106830	Pizza with beans and vegetables, thick crust*
58106910	Pizza with seafood, thin crust*
58106915	Pizza with seafood, regular crust*
58107220	White pizza, thin crust*
58107225	White pizza, regular crust*
58107230	White pizza, thick crust*
58108000	Calzone, with cheese, meatless*
58108010	Calzone, with meat and cheese*
58108050	Pizza rolls*
58116115	Empanada, Mexican turnover, filled with cheese and vegetables*

Food Code	Description
58116310	Cheese turnover, Puerto Rican style (Pastelillo de queso; Empanadilla)*
58117110	Commeal fritter, Puerto Rican style (Arepa; P.R. arepita)*
58120110	Crepes, filled with meat, fish, or poultry, with sauce*
58122210	Gnocchi, cheese*
58124210	Pastry, cheese-filled*
58124250	Spanakopitta*
58125110	Quiche with meat, poultry or fish*
58125120	Spinach quiche, meatless*
58125180	Cheese quiche, meatless*
58126290	Turnover, meat- and cheese-filled, lower in fat*
58126300	Turnover, meat- and cheese-filled, tomato-based sauce, lower in fat*
58126400	Turnover, filled with egg, meat and cheese*
58127150	Vegetables and cheese in pastry*
58127210	Croissant sandwich, filled with ham and cheese*
58130011	Lasagna with meat*
58130013	Lasagna with meat, canned*
58130020	Lasagna with meat and spinach*
58130140	Lasagna with chicken or turkey*
58130150	Lasagna, with chicken or turkey, and spinach*
58130310	Lasagna, meatless*
58130320	Lasagna, meatless, with vegetables*
58131100	Ravioli, NS as to filling, no sauce*
58131110	Ravioli, NS as to filling, with tomato sauce*
58131120	Ravioli, NS as to filling, with cream sauce*
58131310	Ravioli, meat-filled, no sauce*
58131320	Ravioli, meat-filled, with tomato sauce or meat sauce*
58131330	Ravioli, meat-filled, with cream sauce*
58131510	Ravioli, cheese-filled, no sauce*
58131520	Ravioli, cheese-filled, with tomato sauce*
58131530	Ravioli, cheese-filled, with meat sauce*
58131535	Ravioli, cheese-filled, with cream sauce*
58131590	Ravioli, cheese and spinach-filled, no sauce*
58131600	Ravioli, cheese and spinach-filled, with cream sauce*
58131610	Ravioli, cheese and spinach filled, with tomato sauce*
58133110	Manicotti, cheese-filled, no sauce*
58133120	Manicotti, cheese-filled, with tomato sauce, meatless*
58133130	Manicotti, cheese-filled, with meat sauce*
58133140	Manicotti, vegetable- and cheese-filled, with tomato sauce, meatless*
58134110	Stuffed shells, cheese-filled, no sauce*
58134120	Stuffed shells, cheese-filled, with tomato sauce, meatless*
58134130	Stuffed shells, cheese-filled, with meat sauce*
58134160	Stuffed shells, cheese- and spinach- filled, no sauce*

Food Code	Description
58134620	Tortellini, cheese-filled, meatless, with tomato sauce*
58134623	Tortellini, cheese-filled, meatless, with tomato sauce, canned*
58134640	Tortellini, cheese-filled, meatless, with vinaigrette dressing*
58134650	Tortellini, meat-filled, no sauce*
58134660	Tortellini, cheese-filled, with cream sauce*
58134680	Tortellini, cheese-filled, no sauce*
58134710	Tortellini, spinach-filled, with tomato sauce*
58134720	Tortellini, spinach-filled, no sauce*
58134810	Cannelloni, cheese- and spinach-filled, no sauce*
58145113	Macaroni or noodles with cheese, canned*
58145115	Macaroni or noodles with cheese, from boxed mix with already prepared cheese sauce*
58146120	Pasta with cheese and meat sauce*
58146130	Pasta with carbonara sauce*
58146150	Pasta with cheese and tomato sauce, meatless*
58147100	Pasta with pesto sauce*
58147330	Macaroni, creamed, with cheese*
58147340	Macaroni, creamed, with cheese and tuna*
58148180	Macaroni or pasta salad with cheese*
58155610	Rice meal fritter, Puerto Rican style (Almojabana)*
58161110	Rice casserole with cheese*
58161120	Brown rice casserole with cheese*
58162090	Stuffed pepper, with meat*
58162110	Stuffed pepper, with rice and meat*
58162120	Stuffed pepper, with rice, meatless*
58163330	Flavored rice mixture with cheese*
58200300	Wrap sandwich, filled with meat, poultry, or fish, vegetables, rice, and cheese*
58301020	Lasagna with cheese and sauce (diet frozen meal)*
58301030	Veal lasagna (diet frozen meal)*
58301050	Lasagna with cheese and meat sauce (diet frozen meal)*
58301080	Lasagna with cheese and meat sauce, reduced fat and sodium (diet frozen meal)*
58301110	Vegetable lasagna (frozen meal)*
58301150	Zucchini lasagna (diet frozen meal)*
58302000	Macaroni and cheese (diet frozen meal)*
58302050	Beef and noodles with meat sauce and cheese (diet frozen meal)*
58302080	Noodles with vegetables in tomato-based sauce (diet frozen meal)*
58303100	Rice, with broccoli, cheese sauce (frozen side dish)*
58304010	Spaghetti and meatballs dinner, NFS (frozen meal)*
58304050	Spaghetti with meat and mushroom sauce (diet frozen meal)*
58304200	Ravioli, cheese-filled, with tomato sauce (diet frozen meal)*
58304220	Rigatoni with meat sauce and cheese (diet frozen meal)*

Food Code	Description
58304230	Ravioli, cheese-filled, with vegetable and fruit (frozen meal)*
58305250	Pasta with vegetable and cheese sauce (diet frozen meal)*
58306010	Beef enchilada dinner, NFS (frozen meal)*
58306020	Beef enchilada, chili gravy, rice, refried beans (frozen meal)*
58306070	Cheese enchilada (frozen meal)*
58306100	Chicken enchilada (diet frozen meal)*
58421080	Sopa de tortilla, Mexican style tortilla soup*
71301020	White potato, cooked, with cheese*
71301120	White potato, cooked, with ham and cheese*
71405100	White potato, hash brown, with cheese*
71410500	White potato skins, with adhering flesh, fried, with cheese*
71411000	White potato skins, with adhering flesh, fried, with cheese and bacon*
71501070	White potato, from dry, mashed, made with milk, fat, egg and cheese*
71507040	White potato, stuffed, baked, peel not eaten, stuffed with broccoli and cheese sauce*
71508040	White potato, stuffed, baked, peel eaten, stuffed with broccoli and cheese sauce*
71508120	White potato, stuffed with ham, broccoli and cheese sauce, baked, peel eaten*
71801100	Potato and cheese soup*
72116140	Caesar salad (with romaine)*
72125250	Spinach, cooked, NS as to form, with cheese sauce*
72125251	Spinach, cooked, from fresh, with cheese sauce*
72125252	Spinach, cooked, from frozen, with cheese sauce*
72125253	Spinach, cooked, from canned, with cheese sauce*
72201230	Broccoli, cooked, NS as to form, with cheese sauce*
72201231	Broccoli, cooked, from fresh, with cheese sauce*
72201232	Broccoli, cooked, from frozen, with cheese sauce*
73102252	Carrots, cooked, from frozen, with cheese sauce*
73305010	Squash, winter, baked with cheese*
75140500	Broccoli salad with cauliflower, cheese, bacon bits, and dressing*
75143200	Lettuce, salad with cheese, tomato and/or carrots, with or without other vegetables, no dressing*
75143350	Lettuce salad with egg, cheese, tomato, and/or carrots, with or without other vegetables, no dressing*
75145000	Seven-layer salad (lettuce salad made with a combination of onion, celery, green pepper, peas, mayonnaise, cheese, eggs, and/or bacon)*
75146000	Greek Salad*
75400500	Artichokes, stuffed*
75401010	Asparagus, NS as to form, creamed or with cheese sauce*
75401011	Asparagus, from fresh, creamed or with cheese sauce*
75401012	Asparagus, from frozen, creamed or with cheese sauce*
75403010	Beans, string, green, NS as to form, creamed or with cheese sauce*
75403011	Beans, string, green, from fresh, creamed or with cheese sauce*

Food Code	Description
75403012	Beans, string, green, from frozen, creamed or with cheese sauce*
75403013	Beans, string, green, from canned, creamed or with cheese sauce*
75409010	Cauliflower, NS as to form, creamed*
75409011	Cauliflower, from fresh, creamed*
75409012	Cauliflower, from frozen, creamed*
75409020	Cauliflower, batter-dipped, fried*
75410500	Chiles rellenos, cheese-filled (stuffed chili peppers)*
75410530	Chiles rellenos, filled with meat and cheese (stuffed chili peppers)*
75410550	Jalapeno pepper, stuffed with cheese, breaded or battered, fried*
75412060	Eggplant parmesan casserole, regular*
75412070	Eggplant with cheese and tomato sauce*
75414020	Mushrooms, stuffed*
75416600	Pea salad with cheese*
75418040	Squash, summer, casserole, with cheese sauce*
75440500	Vegetable combinations (including carrots, broccoli, and/or dark-green leafy), cooked, with cheese sauce*
75440510	Vegetable combinations (excluding carrots, broccoli, and dark-green leafy), cooked, with cheese sauce*
75608100	Onion soup, French*
76102030	Broccoli, carrots and cheese, baby food, junior*
77316600	Eggplant and meat casserole*
81302070	Pesto sauce*
83103500	Feta Cheese Dressing*

* Only component of proposed food category of food was applied in analysis

Foods included for proposed use analysis:

Food Code	Description
Yogurt products	
11410000	Yogurt, NS as to type of milk or flavor
11411010	Yogurt, plain, NS as to type of milk
11411100	Yogurt, plain, whole milk
11411200	Yogurt, plain, lowfat milk
11411300	Yogurt, plain, nonfat milk
11420000	Yogurt, vanilla, lemon, or coffee flavor, NS as to type of milk
11421000	Yogurt, vanilla, lemon, or coffee flavor, whole milk
11422000	Yogurt, vanilla, lemon, maple, or coffee flavor, lowfat milk
11422100	Yogurt, vanilla, lemon, maple, or coffee flavor, lowfat milk, sweetened with low calorie sweetener
11423000	Yogurt, vanilla, lemon, maple, or coffee flavor, nonfat milk
11424000	Yogurt, vanilla, lemon, maple, or coffee flavor, nonfat milk, sweetened with low calorie sweetener
11425000	Yogurt, chocolate, NS as to type of milk
11426000	Yogurt, chocolate, whole milk
11430000	Yogurt, fruit variety, NS as to type of milk
11431000	Yogurt, fruit variety, whole milk
11432000	Yogurt, fruit variety, lowfat milk
11432500	Yogurt, fruit variety, lowfat milk, sweetened with low-calorie sweetener
11433000	Yogurt, fruit variety, nonfat milk
11433500	Yogurt, fruit variety, nonfat milk, sweetened with low-calorie sweetener
11446000	Fruit and lowfat yogurt parfait
27516010	Gyro sandwich (pita bread, beef, lamb, onion, condiments), with tomato and spread*
41420380	Soy yogurt
51108100	Naan, Indian flatbread*
53104580	Cheesecake -type dessert, made with yogurt, with fruit*
53441210	Basbousa (semolina dessert dish)*
58124500	Pastry, filled with potatoes and peas, fried*
83115000	Yogurt dressing*

* Only component of proposed food category of food was applied in analysis

Annex IX-1

JECFA Safety Evaluation of certain food additives and contaminants,
Natamycin

WHO FOOD ADDITIVES SERIES: 48, 2002

Authors

Dr A. Mattia

Dr C. Cerniglia

J. Baines

SAFETY EVALUATION OF CERTAIN FOOD ADDITIVES AND CONTAMINANTS

NATAMYCIN (PIMARICIN)

First draft prepared by Dr Antonia Mattia¹, Dr Carl Cerniglia² and Janis Baines³

¹Division of Product Policy, Office of Premarket Approval, Center for Food Safety and Applied Nutrition, Food and Drug Administration, Washington DC 20204, USA;

²Division of Microbiology, National Center for Toxicological Research, Food and Drug Administration, Jefferson, Arkansas, USA;

³Monitoring and Evaluation Program, Australia New Zealand Food Authority, Canberra, Australia

Explanation

Biological data

Biochemical aspects: Absorption, distribution, and excretion

Toxicological studies

Acute toxicity

Short-term studies of toxicity

Long-term studies of toxicity and carcinogenicity

Genotoxicity

Reproductive toxicity

Multigeneration studies

Developmental toxicity

Special studies

Allergic effects

Degradation products

Acute toxicity

Short-term studies of toxicity

Microbiological effects

Mechanism of action

Fungi in the human gastrointestinal tract

Fungal resistance to natamycin
Observations in humans
Intake
Screening for additives by the budget method
Individual dietary records
Evaluation of intake estimates
Comments
Evaluation
References

1. EXPLANATION

Natamycin (pimaricin) is a polyene macrolide antibiotic produced by submerged aerobic fermentation of *Streptomyces natalensis* and related species. Fermentation is conducted for several days, and the antibiotic is isolated either by broth extraction or by extraction of the mycelium. It is used as a food additive to control the growth of yeasts and moulds on the surface of cheese and other non-sterile products, such as meat and sausages.

Natamycin was evaluated by the Committee at its twelfth and twentieth meetings (Annex 1, references 17 and 41). At its twentieth meeting, the Committee established an ADI of 0–0.3 mg/kg bw. Natamycin was evaluated at the present meeting at the request of the Codex Committee on Food Additives and Contaminants.

The Committee considered information on the current uses of natamycin, biological data not previously evaluated, and data on its intake.

The activity of natamycin against yeasts and moulds, but not bacteria, makes it convenient for use in foods that undergo a ripening period after processing. Its low solubility in water and most organic solvents makes it suitable for the surface treatment of foods. Natamycin is used topically in veterinary medicine to treat mycotic infections, such as ringworm in cattle and horses. Previously, it was used topically against fungal infections of the skin and mucous membranes in humans. Its medical use is now confined to topical treatment of corneal fungal infections and the prevention of such infections in users of contact lens.

2. BIOLOGICAL DATA

2.1 Biochemical aspects: Absorption, distribution, and excretion

Rats

The distribution of natamycin was studied by autoradiographic and bioautographic techniques. In the autoradiographic study, five female Wistar rats (TNO, specific pathogen-free) were each given a single dose of 50 mg/kg bw of [¹⁴C]natamycin (50 mg in 5 ml of 1% amyllum) orally. In the bioautographic study, four female rats were each given a single dose of 50 mg/kg bw (70 mg in 7 ml of 1% amyllum) orally. No information on the purity of the compound was provided. Before treatment, the animals were fasted for 24 h but were given a 5% glucose drinking-water solution. One animal in each group was killed by immersion in a solid CO₂ and acetone mixture under mild ether anaesthesia 1, 2 (autoradiographic study only), 4, 8, and 24 h after treatment. Whole-body sections of the animals were cut in a cryostat at -20 °C. In the autoradiographic study, sections were freeze-dried (48 h) and exposed on photographic plates at -20 °C for 93 days (a few for 150 days). In the bioautographic study, the antibiotic activity of the sections was evaluated by exposure on Whiffen agar plates inoculated with *Saccharomyces cerevisiae* strain ATCC 9763 for 5, 10, 15, or 20 min (20, 40, 60, and 120 min for sections from the animals killed 24 h after treatment). After exposure, the agar plates were incubated at 30 °C for 20 h and photographed.

In the autoradiographic study, radiolabel was confined to the gastrointestinal tract after 93 days' exposure (1 h, oesophagus, stomach, small intestine; 2 h, oesophagus, stomach, small intestine, caecum; 4 h, stomach, small intestine, caecum, colon; 8 h, stomach, intestine; 24 h, caecum, colon). After 150 days' exposure, radiolabel was visible only faintly after magnification of the pictures, in the liver, kidneys, and fatty tissue, in addition to the gastrointestinal tract. In the bioautographic study, the antibiotic activity of natamycin was restricted to the gastrointestinal tract (1 h, stomach, small intestine; 4 h, stomach, small intestine, caecum; 8 h, stomach, small intestine, caecum) and lasted less than 24 h. No antibiotic activity was observed in the colon. The results of the autoradiographic study indicate that natamycin is minimally absorbed into the bloodstream and excreted almost entirely in the faeces. The lack of antibiotic activity and the presence of radiolabel in the caecum and colon 24 h after dosing are consistent with the breakdown of natamycin into microbiologically inactive compounds by bacterial flora in the caecum and colon (Blankwater & Hespe, 1979).

A series of experiments was conducted to study the excretion and resorption of [¹⁴C]natamycin and its degradation products in normal and cholestatic Wistar rats (induced by tying the bile ducts with a ligature). In the first series, the excretion pattern of radiolabelled compound was investigated in groups of three young male Wistar rats given [¹⁴C]natamycin at a dose of 0.1, 1, or 10 mg/kg by quantifying the amount of radiolabel in the urine and faeces at 24-h intervals for 72 h and in expired breath hourly for up to 7 h. Another group received a single dose of 10 mg/kg bw intraperitoneally. A similar experiment was performed in which 10 mg/kg bw of the degradation products of [¹⁴C]natamycin, obtained by acid hydrolysis to simulate that in the stomach, were administered orally or intraperitoneally to three Wistar rats, and their urine, faeces, and expired breath were analysed as described above.

Separate experiments were conducted to determine the elimination of [^{14}C]-natamycin in the bile by giving 10 mg/kg bw to two rats orally and to four rats by intraperitoneal injection. Bile was obtained via a cannula in the bile duct at 1-h intervals for 7 h and analysed for radiolabel. In a similar experiment, the elimination of [^{14}C]natamycin via the bile was determined after oral administration of 10 mg/kg bw, in which 0.1 ml of bile obtained from rats not treated with natamycin was placed in the duodenum of treated animals. Bile was collected hourly for 7 h and analysed for radiolabel.

A series of analyses was also carried out to quantify the radiolabel in the stomach, small intestine, caecum, and large intestine of groups of two animals 1, 2, 4, 8, and 24 h after administration of 10 mg/kg bw [^{14}C]natamycin. Sections of the stomach, small intestine, caecum, and large intestine were extracted in methanol, and the extracts were analysed for radiolabel by thin-layer chromatography. One rat was given the non-radioactive form of the test material and killed after 4 h. The concentration of natamycin was analysed in each section of the gastrointestinal tract by high-performance liquid chromatography. The results were compared with those obtained with the radioactive form. In each experiment, all animals were fasted for 20 h before treatment. Water was available during fasting.

When 10 mg/kg bw [^{14}C]natamycin were administered orally to normal or cholestatic rats, most of the radiolabel (93–103%) was found in the faeces. Cholestatic rats had about 5% more radiolabel in their urine than normal rats at this dose. The results were similar in rats treated with 0.1 or 1 mg/kg bw. When natamycin was delivered by intraperitoneal injection at a dose of 10 mg/kg bw, about 16% of the radiolabel was found in urine and about 76% in faeces by 72 h, indicating significant elimination in the bile. Most of the elimination (63%) occurred within 24 h after administration of natamycin. Intraperitoneal administration of acid-hydrolysed [^{14}C]natamycin resulted in approximately twice as much radiolabel in the urine (61%) as in faeces (30%), showing that hydrolysis transforms natamycin into breakdown products which are more hydrophilic than intact natamycin and thus have less affinity for bile. In contrast, after oral administration of acid-hydrolysed [^{14}C]natamycin, most of the radiolabel was recovered in faeces (94% as compared with 6.7% in urine); thus, hydrolysis did not appear to result in significant systemic absorption. Little radiolabel associated with either intact or acid-hydrolysed natamycin was eliminated as $^{14}\text{CO}_2$ in expired breath (< 1%) after either oral or intraperitoneal administration.

In experiments to determine the amount of radiolabel in bile after an oral or intraperitoneal dose of 10 mg/kg bw [^{14}C]natamycin, 40% of the total radiolabel was recovered over 7 h from the bile of rats treated intraperitoneally and only 1% from the bile in rats treated orally. When 'blank' bile was administered duodenally each hour for 7 h to animals treated orally, the amount of radiolabel recovered in the bile was similar to that recovered in animals not given bile.

In the stomach and small intestine, natamycin was mostly untransformed, as indicated by thin-layer chromatography. Most degradation took place in the large intestine. The degradation products were more hydrophobic than natamycin and were found from about 4 h after treatment. Most of the dose of 10 mg/kg was degraded about 8 h after treatment, suggesting that elimination is relatively rapid. Biotransformation was attributed to the bacterial flora in the caecum and small intestine.

Overall, no more than 5–7% of the total radioactive dose was absorbed after oral administration of [^{14}C]natamycin, and approximately 90% of the administered compound passed through the gastrointestinal tract without resorption and was eliminated in the faeces (Meier & Hespe, 1979).

Dogs

The resorption and excretion of natamycin were studied by autoradiography in dogs given the compound in plastic coating on cheese at 0.75–0.88 mg/kg, in gelatin capsules at 1.00–1.03 mg/kg, or in a 1% starch suspension at 0.95–1.0 mg/kg. In another experiment, [¹⁴C]natamycin was administered intravenously in 5 ml propylene glycol at a concentration of 1 mg/ml. In the experiments with cheese, a radioactive plastic coating with a natamycin content of approximately 2% was applied to one side of 20-g blocks of cheese. A single batch of natamycin was labelled with ¹⁴C by incorporating labelled sodium acetate as the substrate in the usual fermentation process. The quantity of applied radioactive natamycin was determined by weighing the blocks of cheese before and after application of the radioactive plastic coating. Tests were carried out with blocks that had been stored at 4 °C for various periods. Four female beagle dogs, weighing 10–12.5 kg, were used in these experiments. Three of the four dogs were used in multiple tests, but at least 2 weeks were allowed to elapse between experiments to ensure complete elimination of radiolabelled material from the previous experiment. Before dosing, the animals were fasted for about 16 h but were given drinking-water. The animals were housed individually in metabolism cages after dosing, and faeces and urine were collected daily for 2–5 days. The samples were processed appropriately, and radiolabel was measured with a liquid scintillation counter. The plastic coating was analysed by thin-layer chromatography to quantify natamycin and reaction products formed during storage.

After oral administration, most of the radiolabel was eliminated in the faeces within 24 h, with less than 4% of the total dose in urine. This pattern of excretion was consistent with all three forms of orally administered natamycin. Storage of the cheese at 4 °C for various lengths of time (1–57 days) had no effect on the pattern of radiolabel observed. Thin-layer chromatographic analysis confirmed the presence of [¹⁴C]natamycin in the cheese coating after up to 56 days at 4 °C. Approximately equal amounts of radiolabel were measured in faeces and urine after intravenous administration of natamycin in propylene glycol, suggesting that resorption occurred via biliary elimination. The amount of radiolabel recovered was < 100% after administration in an oral capsule or a suspension and was > 100% after administration as a cheese coating. This difference was probably due to uncertainties in the experimental procedure. It is not clear from this study if excretion of natamycin in the faeces after oral administration was due to the lack of absorption from the gastrointestinal tract or to resorption of systemic natamycin via bile (Hespe & Meier, 1980).

Humans

Little information was available on the absorption, distribution, excretion, or metabolism of natamycin in humans. No natamycin (< 1 µg/ml) could be detected in the blood after ingestion of 500 mg by human subjects (Anonymous, 1968). This finding corroborates the statement that natamycin is not absorbed from the gut in animals or humans (Raab, 1972).

2.2 Toxicological studies

2.2.1 Acute toxicity

The available data on the acute toxicity of natamycin are summarized in Table 1. They suggest that male rats are more sensitive to the acute toxicity of orally administered natamycin than females (Levinskas et al., 1966). However, in a study by van Eken and Wubs (1976) to determine the LD₅₀ values for natamycin and three of its potential metabolites after intraperitoneal administration to mice, females were more susceptible to the lethal effects (Table 1). The LD₅₀ values of the metabolites of natamycin in this study were higher than that for natamycin, indicating that they are less acutely toxic than the parent compound. LD₅₀ values of 3200, 3700, and > 4000 mg/kg were reported for aponatamycin (*n* = 2), mycosamine hydrochloride (*n* = 2), and dinatamycinolidediole (range-finding study), respectively, which are potential metabolites of natamycin.

Table 1. Acute toxicity of natamycin

Species	Sex	Route	LD50 (g/kg bw)	Reference
Mouse	NR	Oral	1500 2500	Anonymous (1965)
Rat	Male Female	Oral	2700 4700	Levinskas et al. (1966)
Guinea-pig	Female	Oral	450	Struyk et al. (1958)
Rabbit	Male	Oral	1400	Levinskas et al. (1966)
Dog	NR	Oral	1000	Anonymous (1965)
Mouse	Male Female	Intraperitoneal	1600 420	van Eeken & Wubs (1976)

NR, not reported

In rabbits, doses of natamycin \geq 500 mg/kg bw caused diarrhoea, and the animals that died had haemorrhagic gastric mucosa. Complexing of natamycin with one-third its weight of a modified polysaccharide increased its toxicity sixfold, and when it was fed to rats natamycin was detected in their blood (Raab, 1972).

2.2.2 Short-term studies of toxicity

Rats

Oral administration of natamycin at doses of 50–70 mg/kg bw per day for 5–10 weeks had no effect on the growth, blood, or tissues of rats. A daily oral dose of 150 mg/kg bw for 9 weeks caused some growth inhibition, and a daily dose of 500 mg/kg bw caused 30% of the rats to die within 2 weeks (Struyk, 1958).

Groups of 20 male and 20 female rats were fed diets containing natamycin at a concentration of 0, 125, 500, 2000, or 8000 mg/kg for 94–96 days. None of the five deaths observed could be attributed to treatment. Growth was retarded and food intake was diminished at the two highest concentrations. The results of haemato-logical examinations and organ weights were within normal limits, and no gross or microscopic lesions were found that could be attributed to natamycin (Levinskas et al., 1966).

Dogs

Groups of three male and three female beagle dogs received diets containing natamycin at a concentration of 0, 125, 250, or 500 mg/kg for 2 years. All but one dog that receiving 250 mg/kg survived for 2 years; the death was unrelated to exposure to natamycin. No effect was seen on food intake, but males receiving the highest concentration did not grow as rapidly as controls initially, and after 15 months, when the dietary intake was reduced, some animals were unable to maintain a satisfactory body weight. The results of haematological and clinical chemical studies revealed no abnormalities. No effects of significance were found on organ weights, and gross and microscopic examination showed no pathological changes (Levinskas et al., 1966).

Groups of two male and two female beagle dogs were given diets containing natamycin at a target concentration of 0, 375, or 750 mg/kg (equivalent to 0, 12, and 25 mg/kg bw per day) for 3 months. The natamycin was obtained in micronized form and was 90.5% pure. The animals were monitored for clinical changes, body weight, food consumption, haematological, clinical chemical, and urinary alterations, electrocardiography (wave intervals and heart rate at weeks 0, 4, 8, and 12), ophthalmology, and pupillary reactions. After being killed by an intravenous overdose of pentobarbital, all animals were necropsied, and the weights of the thymus, heart, liver, kidneys, adrenals, spleen, and testes were measured and gross lesions noted. The tissues preserved in buffered formaldehyde saline and examined microscopically were brain, thyroid, thymus, lung, heart, liver, kidneys, adrenals, spleen, pancreas, lymph nodes, urinary bladder, ovaries, testes, stomach, ileum, colon, jejunum, caecum, and oesophagus. The statistical evaluations included analysis of variance and the Student *t* test. A signed statement indicated that the study had been conducted in compliance with regulations for Good Laboratory Practice as specified in the Code of Federal Regulations (Title 21, part 58) of the USA and the OECD. A signed and dated quality assurance statement indicated that the findings had been audited throughout the study.

No dose- or treatment-related effects were seen in males or females with respect to mortality rate, food consumption, body weight, haematological, clinical chemical, or urinary end-points, electrocardiography, ophthalmology, absolute and relative organ weights, gross pathology, and histopathology. The only effect attributed to treatment was diarrhoea, which occurred most frequently

in animals at the high dose but was also observed in controls and animals at the low dose. The diarrhoea was attributed to local irritation of the gastrointestinal tract. Because of the frequent occurrence of diarrhoea at 750 mg/kg, the authors noted that it would be difficult to expose animals to higher doses. No NOEL could be identified (van Eeken et al., 1984).

2.2.3 Long-term study of toxicity and carcinogenicity

Rats

Groups of 35–40 male and female rats received diets containing natamycin at a concentration of 0, 125, 250, 500, or 1000 mg/kg for 2 years. The animals remained in good health, and their survival was unaffected by treatment. Inhibition of growth rate and diminished food intake were seen only for animals of each sex receiving the highest concentration. The results of haematological investigations and determination of organ weights and gross and microscopic lesions showed no differences between treated and control groups. The numbers and types of tumours found in natamycin-treated rats were not significantly different from those in untreated animals (Levinskas et al., 1966).

2.2.4 Genotoxicity

In vitro

Studies were conducted to evaluate the mutagenic potential of natamycin, its products of degradation (i.e. aponatamycin, natamycinolidediol, and mycosamine hydrochloride), and Delvocid (a 50% suspension of natamycin in water) in *Bacillus subtilis*, *Salmonella typhimurium*, and *Escherichia coli*. *B. subtilis* was exposed in a standard *rec* assay (spot diffusion method) according to Kada (citation not provided). *E. coli* strains WP2 $uvrA^-$ and WP2 and *S. typhimurium* strains TA1535, TA1538, TA98, and TA100 were evaluated in the spot test for reverse mutation. All the tests were carried out by plating a 50- μ l spot containing the appropriate dilution of natamycin on a petri dish with the appropriate microbial strain. The spot tests were carried out within 3 h of exposure and after storage for 1, 3, 7, or 14 days and 1, 2, or 4 months (It was not clear whether all tests were conducted at all intervals). The plate incorporation assay was used to evaluate the mutagenicity of Delvocid at concentrations up to 1% alone (without addition of an exogenous metabolic activation system from rodent liver) and in combination with up to 0.2 mol/L nitrite in *E. coli* WP2 $uvrA^-$ and WP2 *trp* $^-$ and *S. typhimurium* TA98 and TA100, with or without addition of exogenous metabolic activation. Nitrite was tested with Delvocid, as other studies have shown that nitrite in combination with some food preservatives forms reaction products that interact with DNA. The design of these tests is shown in Table 2. In each spot test, negative controls were included with solvent or buffer alone and positive controls with a known mutagen (*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine) or mixtures of sorbic acid and nitrite at pH 3.5 or 4.5. For the plate incorporation assays, benzdine was used as the positive control. No statistical analyses were reported. The author reported that no positive responses were observed in the spot tests in any of the three test systems. Results for individual plates and summary data were not reported for the spot tests. The authors concluded from the plate incorporation assays that Delvocid did not induce reverse mutation when tested alone or with nitrite in any of the strains of *S. typhimurium* or *E. coli* tested. The author commented on the slight positive response with nitrite at about 0.2 mol/L and concluded that Delvocid did not enhance the mutagenic effect (Khoudokormoff, 1977; Khoudokormoff & Gist-Brocades, 1978).

Table 2. Experimental design of study reported by Khoudokormoff (1977)

Material	Concentrations tested (%)	Bacterial system	Metabolic activation	Nitrite concentration
<i>Spot test^a</i>				
Natamycin ^b	0.1–1 ^c	<i>S. typhimurium</i> , <i>E. coli</i> , <i>B. subtilis</i> ^d	No ^e	≤ 400 mg/kg
Aponatamycin	0.5	<i>S. typhimurium</i> , <i>E. coli</i> , <i>B. subtilis</i> ^d	No ^e	≤ 400 mg/kg
Pimaricinolidediol	0.5	<i>S. typhimurium</i> , <i>E. coli</i> , <i>B. subtilis</i> ^d	No ^e	≤ 400 mg/kg
Mycosamine hydrochloride	0.5	<i>S. typhimurium</i> , <i>E. coli</i> , <i>B. subtilis</i> ^d	No ^e	≤ 400 mg/kg
Delvocid ^{b,f} hydrochloride	2	<i>S. typhimurium</i> , <i>E. coli</i> , <i>B. subtilis</i> ^d	No ^e	≤ 400 mg/kg
<i>Plate incorporation assay on top agar</i>				
Delvocid	0.04–1	<i>E. coli</i> , <i>S. typhimurium</i> ^g	No	None
	0.04–1	<i>E. coli</i> , <i>S. typhimurium</i> ^g	No	0.01–0.2 mol/L
	0.4	<i>E. coli</i> , <i>S. typhimurium</i> ^g	Yes	0.01–0.5 mol/L
	0.04–1	<i>E. coli</i> , <i>S. typhimurium</i> ^g	Yes	0.01–0.5 mol/L
				0.5 mol/L

^a Carried out within 3 h of exposure and after storage for 1, 3, 7, or 14 days and 1, 2, or 4 months

^b Tested at pH 2.5–6.5

^c Only range provided

^d *E. coli* strains WP2 *trp*⁻ and WP2*uvrA*⁻ and *S. typhimurium* strains TA1535, TA1538, TA98, and TA100

^e Reported that a metabolic activation system was added ‘if desired’; no further details were provided

^f Also tested in the presence of a cheese coating (WL30) at pH 4.3

^g *E. coli* strains WP2 and WP2*uvrA*⁻ and *S. typhimurium* strains TA98 and TA100

The Committee noted that the reporting of the results of these studies, described as preliminary, had limitations which prevented verification of the author's conclusions. For example, the bacterial strains used were not assayed for the appropriate phenotypic markers or plasmids, the criteria for a positive response were not reported, summary and individual data were not reported for the spot test, and no statistical analyses were performed. Sufficient information was not provided to indicate that the studies were adequately sensitive to detect positive responses in all strains tested. Furthermore, the assays were conducted in a single trial with one plate per dose. The usefulness of these studies is therefore limited.

Natamycin at a concentration of 1% and its known degradation products (apонатamycin, dinatamycinolidediol, and mycosamine) at 0.5% and at pH and nitrite conditions similar to those in preserved food products such as cheese and sausages, were reported to have no mutagenic activity in *B. subtilis* under the conditions tested. No actual data were presented to verify this statement (Khoudokormoff, 1978).

In vivo

Groups of 10 male rats taken from the second litters of the F₁ generation in a three-generation study of reproductive toxicity (see below) were fed on control diet until sexually mature, when they received natamycin at 0, 5, 15, 50, or 100 mg/kg bw daily for 7 days by gastric intubation. Each rat was mated each week for 8 consecutive weeks with two virgin untreated females. Each female was killed and examined 13 days after mating. No differences were found between control and test animals in respect of the numbers of implantation sites or live or dead fetuses or the mutagenic index (Cox et al., 1973).

Five males and five females were selected at random from the five litters produced in the same three-generation study. The animals were given colchicine 3–4 h before being killed, and a bone-marrow preparation was made for examination for aberrant chromatin material. The number of abnormalities in the metaphase chromosomal preparations of test groups did not differ significantly from that in sham-treated controls (Cox et al., 1973).

2.2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

Groups of 10 female and five male rats receiving diets containing natamycin at a concentration of 0 or 1000 mg/kg were mated after 181 and 223 days. Other groups were mated after 48, 174, and 250 days on the diets; four control and four test female young from the second mating were fed on the same diet as their parents and mated when 107 days of age. The pups of natamycin-treated animals had lower mean body weights at weaning than control pups, but examination of the results of the 54 matings showed that their fertility, gestation, lactation, and viability indices were similar to or better than those of the controls. There was a low incidence of abnormalities among pups in this study, but none could be attributed to treatment (Levinskas et al., 1963; Levinskas, 1966).

Groups of 10 male and 20 female rats were given a diet containing natamycin providing a dose of 0 (two groups), 5, 15, 50, or 100 mg/kg bw per day for 11 weeks. These formed the F₀ generation of a three-generation study of reproductive toxicity, two litters being produced in each generation. Animals at 100 mg/kg had an increased number of fetuses born dead, a decrease in the number born alive, and a decrease in the number surviving at 21 days. The weight of pups was depressed in the second litters of the F₀ and F₁ generations and both litters of the F₂ generation. However, the fertility, gestation, viability, and lactation indices were within normal limits for both litters of all three generations. The doses of 5, 15, and 50 mg/kg had no detectable effect on growth or reproduction (Cox et al., 1973).

(b) Developmental toxicity

Rats

Groups of 20 female rats from the second litters of the F₁ generation of the three-generation study of reproductive toxicity were reared to maturity on control diet and mated with untreated males. The females were given the same dose of natamycin as their parents (0, 5, 15, 50, or 100 mg/kg bw per day) by gastric intubation during the 6–15 days of gestation and were killed and examined on day 20. No differences were found between control and test animals in respect of the numbers of pregnancies, live litters, implantation sites, resorption sites, live and dead fetuses, or skeletal and soft tissue abnormalities (Cox et al., 1973).

Rabbits

Groups of 10–12 female rabbits were given natamycin at a dose of 0, 5, 15, or 50 mg/kg bw per day by gavage on days 6–18 of gestation. They were examined on day 29, and the numbers of corpora lutea, implantation sites, resorption sites, and live and dead fetuses were recorded. No adverse effects on nidation or maternal or fetal survival were found. The number of abnormalities seen in the soft or skeletal tissues did not differ from that occurring spontaneously in controls (Bailey & Morgareidge, 1974).

An aqueous suspension of Delvocid (50% natamycin) was administered to groups of 20–26 mated female Dutch belted rabbits by gavage at a dose of 5, 15, or 50 mg/kg bw per day on days 6–18 of gestation. Two control groups were used: a vehicle control that received an equal volume of sterile saline daily by gavage on days 6–18 of gestation and a positive control group given 2.5 mg/kg bw of 6-aminonicotinamide by gavage on day 9 of gestation. The does were observed daily for signs of toxicity, and body weights were recorded on days 0, 6, 9, 12, 15, 18, and 29 of gestation. On day 29, all surviving does were killed, and the numbers of corpora lutea, implantation sites, resorption sites, and live and dead fetuses, sex of fetuses, and fetal body weights were evaluated at autopsy. The survival rate of the fetuses was determined, and they were examined for external, soft-tissue, and skeletal anomalies. According to the protocol, the study was conducted in compliance with proposed Good Laboratory Practice regulations (21 CFR 3), but there is no indication as to whether quality assurance or quality control procedures were in place.

Treated does showed no clinical signs of toxicity. One at the low dose, two at the intermediate dose, and five at the high dose died or were killed when moribund. Accordingly, the maternal mortality rates were 0% (0/20), 5% (1/20), 9% (2/22), and 19% (5/26) in the four groups, respectively. The cause of

these deaths was not indicated in the report. One doe at the intermediate dose delivered young prematurely (the day before scheduled removal). There were no clear treatment-related signs of toxicity. The following parameters were comparable in treated groups and the vehicle control group: mean maternal body weight, pregnancy rate, number of implantation sites, number of resorption sites, numbers of live and dead fetuses, male to female ratio of fetuses, per cent viability, and incidence of soft-tissue anomalies. Maternal body-weight gain was not calculated. In addition, although the number of corpora lutea in each doe and the occurrence of external anomalies were determined, these data were not summarized or analysed statistically. The average body weight of live fetuses in the group at the intermediate dose was significantly lower than that of the vehicle control group. The groups at the two higher doses showed a significant increase relative to the vehicle control group in the incidence of extra sternebrae. The authors noted that the effect on fetal body weight was not dose-related, and they considered the extra sternebrae to be a developmental variation and not an indication of frank teratogenicity (Knickerbocker & Re, 1978, 1979).

The results of this study were difficult to interpret owing to maternal mortality, problems associated with gavage of rabbits, and because the digestive system of rabbits is sensitive to antibiotics. However, there was evidence that the extra sternebrae observed in fetuses of does at the intermediate (15 mg/kg bw per day) and high (50 mg/kg bw per day) doses of natamycin were variations rather than malformations (Manson & Kang, 1994). Consequently, this study was not considered suitable for deriving an ADI.

2.2.6 Special studies

(a) Allergic effects

No allergic sensitization occurred among 111 patients being treated with natamycin for a variety of conditions (Gruyter, 1961, 1964). No history of allergic reactions was found in 73 workers engaged for an average of 5 years in the manufacture of natamycin, and no allergic reactions were found in the 71 who were tested with cutaneous or intradermal challenge doses (Malten, 1967). Repeated patch tests on 102 patients with various forms of eczema failed to demonstrate any sensitizing potential of natamycin (Malten, 1968).

(b) Degradation products

(i) Acute toxicity

Similar breakdown products of natamycin occur in simulated gastric juice, 0.5% citric acid, and urine, and it appears likely that breakdown products in stored apples resemble those produced in gastric juice. The breakdown products are tetraenes related to natamycin, principally aglycone dimerized and/or decarboxylated; whether these are absorbed remains to be tested (Brik, 1975). Approximately 50% natamycin is broken down within 1 h in simulated gastric juice, and the losses from the stomachs of fasted and non-fasted rats were 33–43% and 0–31% respectively (Morgenstern & Muskens, 1975).

The results of studies of the acute toxicity of the decomposition products of natamycin kept under various conditions after intraperitoneal administration to mice are presented in Table 3.

Table 3. Acute toxicity of natamycin decomposition products

Treatment of suspension	Decomposition (%)	LD ₅₀ in mice (mg/kg bw)
pH 2.2 with citric acid	74	200
pH 6.3 in the dark	13	200–400
pH 6.3 in the light	80	400–600
pH 8.5 (NaOH)	0	150–250
pH 8.5 (NaOH)	5	450
pH 10.4 with ‘soda’	100	> 800
pH 6.3 with 0.1% H ₂ O ₂	9	200–400
pH 5.0 in ultra-violet light	0	170

From Ottens (1965)

(ii) Short-term studies of toxicity

Rats

Groups of 15 male and 15 female rats were given diets containing 5% water, 5% of 0.5% citric acid, 500 mg/kg natamycin, or 5% of a solution of acid-degraded natamycin (suspended in 0.5% citric acid until only 14% of the activity remained) for 98 days. No animals died, and their weight gain was unaffected by treatment; no adverse effects were seen in haematological tests or on the absolute weights of the liver and kidneys. Minor differences in relative organ weights were considered to be coincidental and not due to treatment. Microscopic examination of a wide range of organs showed no lesions due to treatment (Hutchison et al., 1966).

Slices of cheese were treated with 0.05% and 5% suspensions of natamycin and left to dry at room temperature. The antimicrobial activity of the two cheeses declined to less than 20% and 60–80% during the 3-week storage period before they were incorporated into rat diet, and the final dietary concentrations of natamycin plus degraded natamycin were 3.6 and 360 mg/kg. Groups of 15 and 30 male and female rats received diets containing fresh cheese dressed with 0, 0.05, or 5.0% natamycin or cheese dressed with 0, 0.05, or 5% suspensions and stored for 3 weeks. The test lasted 7 weeks. No effects that could be attributed to natamycin degradation products were found on behaviour, appearance, morbidity, mortality, food consumption, body-weight gain, haematological indices, liver function, organ weights, or macro- or microscopic appearance of the animals (Wieriks, 1966).

Groups of 10 male and 10 female rats were fed for 3 months on diets containing the peel of apples which had been untreated, freshly treated with natamycin, or treated with natamycin and stored for 2–8 weeks to allow degradation to take place. In a similar experiment, sausage skins untreated, freshly treated, or stored with natamycin were fed to rats. The doses of natamycin and its degradation products cannot be calculated, but the apple-skin diet provided rats with approximately 0, 50, and 1250 times the probable human intake, and the sausage-skin diet provided approximately 0, 1000, and 25 000 times the human intake. Some minor abnormalities were found, but none related to growth rate, mortality rate, haematological indices, serum enzymes, liver function, organ weights, or gross or microscopic appearance could be attributed to the intake of natamycin breakdown products (Wieriks, 1971).

2.2.7 Microbiological effects

Limited information on the microbiological effects of natamycin, including fungal resistance, was included in the previous monograph (Annex 1, reference 42). In that monograph, it was stated that natamycin is active against a wide range of mycotic organisms such as dermatophytes and other fungi, yeasts, and yeast-like organisms (including strains pathogenic to humans, animals, and plants and saprophytic varieties). Standard tests have shown that it has no activity on bacteria or on actinomycetes. There is no evidence that mycotoxin-forming species are unusually resistant to natamycin (Raab, 1972). No yeast or yeast-like organisms have been reported to have primary resistance to natamycin, although some dermatophytes are resistant. It is more difficult to induce resistance to natamycin in yeasts than in bacteria (Khoudokmoff & Petru, 1974), and the resistance that could be obtained appeared to be due to selection of naturally more resistant strains and not to adaptation. The resistant cultures had reduced pathogenicity (Athar & Winner, 1971). No evidence of resistance has been recorded in clinical use of natamycin. In studies of its cross-resistance with other antimicrobials, amphotericin B but not natamycin showed cross-resistance with nystatin, filipin, endomycin, and candidin (Stout & Pagano, 1956; Littman et al., 1958; Bodenhoff, 1968; Walter & Heilmeyer, 1969). Nystatin- and amphotericin-B resistant organisms were susceptible to natamycin (Sørensen et al., 1959), and a wide selection of nystatin-resistant yeasts were normally susceptible to natamycin (Hejzlar & Vymola, 1970). Cross-resistance between natamycin and nystatin and amphotericin appeared to occur *in vitro* (Athar & Winner, 1971).

More information on fungal resistance has become available since the previous review, and that pertinent for assessing potential resistance, including a discussion of the mechanism of action of polyene antibiotics and fungi in the human gastrointestinal tract, is summarized below.

(a) Mechanism of action

The polyenes constitute a large group of antibiotics with various molecular structures, which interact with fungal membranes in an especially interesting way (Franklin & Snow, 1998). The approximately 200 polyenes are all produced by *Streptomyces* spp. The antifungal activities of natamycin and other polyenes are dependent on their binding to cell membrane sterols, primarily ergosterol, the principal sterol in fungal membranes, thereby making them leaky (Hamilton-Miller, 1974; Norman et al., 1976; McGinnis & Rinaldi, 1985; Carlile & Watkinson, 1994). As polyene macrolide antibiotics like amphotericin B, nystatin, and natamycin have a much greater affinity for ergosterol than for cholesterol, the mammalian membrane sterol, they are selectively antifungal. The polyenes form

complexes with sterols and apparently disrupt membrane function by this mechanism. The oomycete fungi and bacteria are insensitive to these antibiotics because their membranes lack sterols. At low concentrations, selective changes in membrane permeability may occur. Leakage of potassium ions is the first detectable event, and, at high concentrations, leakage of amino acids and other metabolites occurs.

The polyenes have a large lactone ring with a rigid lipophilic chain containing three to seven conjugated double bonds and a flexible hydrophilic portion bearing several hydroxyl groups. The length of the chromophore gives the characteristic ultra-violet spectrum for each compound and contributes to the instability of some polyenes to heat, light, and pH. Most polyenes have a sugar unit, typically the amino sugar mycosamine, which is linked by the glycosidic bond to the α carbon atom of the chromophore. Amphotericin B contains seven conjugated double bonds, and natamycin contains four, so these antimicrobial agents are known as heptaenes and tetraenes, respectively. Nystatin is classified as either a pseudoheptaene or a tetraene (McGinnis & Rinaldi, 1985).

The typical polyene structure has both a hydrophobic and hydrophilic face. The polyenes insert themselves into the cell membrane by associating with sterols (the hydrophobic face) and are thought to cause rearrangement of the sterols, so that a group of four to eight polyene molecules forms a ring with the hydrophilic faces in the centre. Thus, they form a polar pore through which small ions like K^+ and H^+ can pass freely, disrupting the cell's ionic control (Griffin 1994; Deacon 1997). Polyenes can also directly affect enzymatic sequences involved in the synthesis of membrane constituents at the level of the early cyclic precursors in the ergosterol biosynthetic pathway (Mukhtar, et al., 1994). The accumulation of these precursors results from a decrease in the *trans*-methylation reaction that requires *S*-adenosylmethione as the donor of the methyl group and zymosterol as the substrate for methylation. Bacteria are not susceptible to natamycin as their membranes are devoid of sterols. Accordingly, the reported minimum inhibitory concentrations (MICs) of natamycin against bacteria are high, those for *Staphylococcus aureus*, *Streptococcus faecalis*, *Streptococcus haemolyticus*, *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhimurium*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* all being > 250 mg/kg.

(b) Fungi in the human gastrointestinal tract

The microflora in the human gastrointestinal tract form an extremely complex, yet relatively stable ecological community, populated with over 10^{11} bacterial cells per gram of content and containing more than 400 bacterial species (Cerniglia & Kotarski, 1999). There are fewer bacteria than fungi. Up to 10^5 colony forming units of yeasts have been reported in stool samples from healthy subjects (Bernhardt, 1998).

The yeasts found are *Candida albicans* (the commonest), *C. glabrata* (*Torulopsis glabrata*), *C. tropicalis*, *C. guilliermondii*, *C. krusei*, *C. inconspicua*, *C. parapsilosis*, *C. lusitaniae*, and *C. kefir* (*C. pseudotropicalis*). *Rhodotorula* spp., *Trichosporon*, *Saccharomyces cerevisiae*, *Geotrichum candidum*, *Aspergillus* spp., *Cryptococcus* spp., and *Mucor* spp. are rarely found in the intestine (Bernhardt, 1998). The metabolic activity of *Candida* spp. in the gastrointestinal tract is very low owing to the anaerobic conditions and limited nutrients. Yeasts of the normal flora can invade the tissues of patients whose immune defences have been suppressed by disease or in persons with an altered intestinal microflora. The therapeutic use of antimicrobials can suppress the normal bacterial flora, and this is

responsible in part for the increase in the number of yeast infections, particularly gastrointestinal candidosis (Blaschke-Hellmessen et al., 1996; Kreisel, 1999). The polyenes are not absorbed from the gastrointestinal tract, but are sometimes given by mouth to combat fungal growth in the gut, which most commonly results from the use of broad-spectrum antibacterials that deplete the normal bacterial flora of the gut and allow yeasts and fungi to multiply and cause opportunistic infection (Scheurle, 1996).

The use of natamycin as an antifungal agent in food may result in trace quantities of antimicrobial residues that interact with endogenous microflora. No data were available on the effect of natamycin on the human intestinal microflora. As bacteria are not affected by polyenes, it can be concluded that natamycin residues would have no potentially harmful effects. Furthermore, as yeasts are found in small quantities in the human gastrointestinal tract, the risk of trace exposure of fungi to natamycin would be minimal.

(c) Fungal resistance to natamycin

Natural resistance against polyenes such as natamycin does not occur among fungi, because of the mode of action of these chemical agents (Khoudokormoff, 1984). Moreover, in contrast with the main polyenes used clinically, such as amphotericin B and nystatin, the fungistatic and fungicidal minimal concentrations of natamycin differ only negligibly (Table 4), further reducing the opportunity for establishment of resistance (Sorensen et al., 1959). Induction of polyene- and especially natamycin-resistant mutants is difficult (Athar & Winner, 1971). Such mutants invariably show reduced metabolic and growth rates *in vitro*, and in the absence of polyenes readily revert to normal metabolism, growth, and sensitivity to natamycin. One way of obtaining such resistant isolates is by successive sub-culturing *in vitro* in the presence of gradually increasing concentrations of a polyene. Typically, such isolates are resistant up to the highest concentration to which they are exposed, and the conditions are not likely to result from technical application of natamycin as a food preservative.

Table 4. Minimal inhibitory concentrations (MICs) of amphotericin B and natamycin when tested in vitro against 28 fungal isolates

Organism	Amphotericin B		Natamycin	
	Mean (µg/ml)	Range (µg/ml)	Mean (µg/ml)	Range (µg/ml)
Group 1 (10 isolates)				
<i>Candida albicans</i>	0.36	0.2–0.6	3.3	1.6–4.7
<i>Candida parapsilosis</i>	2.4	0.8–3.1	5.2	4.7–6.2
<i>Candida krusei</i>	1.6	1.6	1.6	1.6
<i>Rhodotorula</i> spp.	6.2	6.2	2.3	2.3
Group 2 (10 isolates)				
<i>Fusarium solani</i>	20	3.1–50	4.2	3.1–6.2
Group 3 (8 isolates)				
<i>Aspergillus fumigatus</i>	3.1	3.1	3.1	3.1
<i>Aspergillus niger</i>	4.3	2.3–6.2	2.3	1.6–3.1
<i>Penicillium</i> spp.	4.7	4.7	2.3	2.3
<i>Penicillium lilacinus</i>	4.7	4.7	9.4	9.4
<i>Rhizopus</i> spp.	ND	2.3→ 50	9.4	6.2–12
<i>Scopulariopsis brevicaulis</i>	> 50	> 50	3.1	3.1

From Stern (1978)

The antifungal action of polyene antibiotics is based on their linkage with sterols in the cytoplasmic membrane of the fungal cell wall, which distends the wall. The sensitivity of fungal cells to the drug depends on the characteristics of the sterol (Littman et al., 1958; Molzahn & Woods, 1972; Subden et al., 1977). *Candida* strains resistant to nystatin contain more ergosterol than sensitive ones (Athar & Winner, 1971; Safe et al., 1977). Sensitivity to polyene antibiotics is a consistent feature of wild-type fungal strains. Prolonged therapy with an antibiotic results in increased resistance to it. Induced resistance to polyene antibiotics has been observed in *Candida*, *Torulopsis*, and *Cryptococcus* strains (Macura, 1991).

Although there is a potential risk of development of resistance among microbial flora as a consequence of prolonged, repeated application of natamycin, the studies reported indicate that the level of resistance would be low.

Attempts to induce resistance to natamycin in *C. albicans* by serial passage on Sabouraud maltose agar showed that resistance developed gradually. After 25 passages, the MIC was increased from 2.5–12 to 12–50 µg/ml. Comparison of the polyene antibiotics natamycin and fungicidin indicated that strains that are resistant to fungicidin are sensitive to natamycin (Hejzlar & Vymola, 1970; Table 5).

Table 5. Correlation between minimal inhibitory concentrations (MICs) of natamycin and fungicidin in some strains of *Candida* spp.

Strain	MIC (µg/ml)	
	Natamycin	Fungicidin
<i>Candida albicans</i> 1	10	5.0
<i>albicans</i> 2	6.2	> 100
<i>albicans</i> 135	5.0	12
<i>albicans</i> 32/V	3.1	3.1
<i>Candida crusei</i> 182	10	6.2
<i>crusei</i> 196	10	3.1
<i>crusei</i> 87/V	10	12
<i>Candida tropicalis</i> 11	6.2	10
<i>tropicalis</i> 94	5.0	12
<i>tropicalis</i>	3.1	> 100
<i>Candida albicans</i> 36/V	2.5	> 100
<i>albicans</i> 69/V	5.0	> 100
<i>albicans</i> 101/V	6.2	1.6
<i>albicans</i> 137/V	> 100	6.2
<i>albicans</i> 138/V	12	12
<i>albicans</i> 141/V	6.2	> 100

<i>albicans</i>	12	3.1
<i>albicans</i> 165/V	12	2.1

From Hejzlar & Vymola (1970)

Natamycin has been given orally for the treatment of intestinal candidosis at a daily dose of up to 400 mg. It was highly active against yeast-like fungi (MIC, 1.5 µg/ml) but less effective against dermatophytes (MIC, 3.0–100 µg/ml). Strains resistant to natamycin are rare, but the effectiveness of this drug in the treatment of vaginal candidosis has decreased (Lovgren & Salmela, 1978). The MIC values were between 2.9 and 31 µg/ml for strains isolated from untreated women but 9.8–64 µg/ml for strains from women who had been treated previously.

Delvocid, a 50% natamycin preparation, has been used for more than 20 years for preserving cheese and sausages (Jay, 1996). Surveys in cheese warehouses and in dry sausage factories where Delvocid had been used for up to 9 years showed no change in the composition or the sensitivity of the contaminating fungal flora (de Boer & Stolk-Horsthuis, 1977; de Boer, 1979; Hoekstra & Van der Horst, 1998).

de Boer and Stolk-Horsthuis (1974) isolated yeasts and moulds from various cheese warehouses in which natamycin was used. All of the isolated fungi but one were inhibited at low concentrations of natamycin (0.5–8 µg/ml). In a similar study in 1976, in which eight warehouses where natamycin had been used and two in which it had never been used were surveyed, 26 strains were isolated and tested for sensitivity to natamycin; no insensitive yeasts or moulds were found.

Laboratory experiments intended to induce tolerance to natamycin in strains isolated from cheese warehouses indicated that after 25–30 transfers to media containing increasing concentrations of natamycin none of the strains had become less sensitive to natamycin (Table 6).

Table 6. Induction of tolerance to natamycin of moulds isolated from cheese warehouses

Strain	No. of transfers	MIC of natamycin (µg/ml)	
		Initial	After a given no. of transfers
<i>Penicillium viridicatum</i> Westling	30	8	10
<i>Penicillium viridicatum</i> Westling	30	8	10
<i>Penicillium chrysogenum</i> Westling	31	2	2
<i>Aspergillus versicolor</i> (Vuill.) Tiraboschi	25	4	1

<i>Penicillium viridicatum</i> Westling	30	8	12
<i>Cladosporium cladosporioides</i> (Fres.) de Vr.	30	2	2
<i>Aspergillus versicolor</i> (Vuill.) Tiraboschi	25	4	2
<i>Penicillium verrucosum</i> Dierckx var. <i>cyclopium</i> (Westling)	31	2	2
<i>Aspergillus versicolor</i> (Vuill.) Tiraboschi	25	2	1
<i>Penicillium viridicatum</i> Westling	29	6	10
<i>Penicillium verrucosum</i> Dierckx var. <i>cyclopium</i> (Westling)	31	2	2
<i>Penicillium verrucosum</i> Dierckx var. <i>cyclopium</i> (Westling)	31	2	2
<i>Cladosporium cladosporioides</i> (Fres.) de Vr.	27	2	6
<i>Penicillium citreo-viride</i> Biourge	28	4	4
<i>Penicillium verrucosum</i> Dierckx var. <i>cyclopium</i> (Westling)	25	4	2
<i>Penicillium brevi-compactum</i> Dierckx	30	4	2
<i>Beauveria alba</i> (Limber) Saccas	23	8	4
<i>Penicillium roseo-purpureum</i> Dierckx	29	8	10
<i>Scopulariopsis asperula</i>	25	8	4
<i>Penicillium cf. lividum</i> Westling	25	4	2
<i>Aspergillus versicolor</i> (Vuill.) Tiraboschi	25	4	2
<i>Acremomium sclerotigenum</i>	23	8	8
<i>Penicillium viridicatum</i> Westling	30	8	10

<i>Penicillium viridicatum</i> Westling	29	8	12
<i>Penicillium nigricans</i> (Bain.) Thom	30	4	2
<i>Aspergillus versicolor</i> (Vuill.) Tiraboschi	25	2	2

From De Boer & Stolk-Horsthuis (1977)

The sensitivity to natamycin of yeasts and moulds isolated in dry sausage factories where natamycin had been used for several years was compared with that of isolates from factories where natamycin had never been applied. No significant differences were found (de Boer et al., 1979).

In experiments with the plant pathogens *Cladosporium cucumerinum* and *Fusarium oxysporum*, the frequency of emergence of resistance was 1 in 10⁷. Eighteen resistant strains were selected for further study, and the natamycin-resistant strains were divided into those with lower and higher levels of resistance. Greater resistance appeared to be associated with decreased fitness *in vitro* (radial growth and sporulation on agar media) and *in vivo* (pathogenicity). The authors suggested a link between increased resistance and decreased fitness (Dekker & Gielink, 1979).

Reduced sensitivity to polyenes can be induced *in vitro*, but this may be of no practical significance. The resistance of several subcultures in the presence of increasing concentrations of a polyene antimycotic was associated with slower growth and diminished virulence, so that any resistant cells that appear during polyene antimycotic treatment may succumb to the body defence mechanisms.

2.3 Observations in humans

Nausea, vomiting, and diarrhoea have been observed occasionally after an oral dose of 300–400 mg of natamycin daily; no changes in peripheral blood cells were observed (Anonymous, 1966). A group of 10 patients with systemic mycoses received oral doses of 50–1000 mg/day for 13–180 days. Nausea, vomiting, and diarrhoea occurred in those receiving 600–1000 mg/day (Newcomer et al., 1960).

3. INTAKE

Natamycin is proposed in the Codex draft General Standard for Food Additives (GSFA) for use in food groups 1.6 ‘Cheese’ at 40 mg/kg, in 8.2.1.2 ‘Cured and dried non-heat treated processed meats, poultry and game products’ at 6 mg/kg, and in 8.3.1.2 ‘Cured and dried non-heat comminuted meat, poultry and game products’ at 20 mg/kg.

Data on intake of natamycin were submitted by Australia, Germany, New Zealand, and by the manufacturer, whose submission included limited data on intake in the United Kingdom and the USA.

3.1 Screening of additives by the budget method

The budget method can be used to assess whether the use of natamycin should be restricted to specific food groups. The calculations indicated that the theoretical maximum level of use of natamycin is 24 mg/kg, assuming it is used in only half the solid food supply and that the ADI is 0–0.3 mg/kg bw. This theoretical level is lower than the proposed level of use in cheese in the draft GSFA but higher than that proposed for meats, indicating that use of natamycin should be restricted.

As natamycin is proposed for use in two single food groups, the reverse budget method can indicate the maximum amount of each food group that can be consumed before the ADI is exceeded, assuming use in only one food group. If use is assumed to be only in cheese, up to 450 g could be consumed per day at a concentration of 40 mg/kg, assuming an ADI of 0–0.3 mg/kg bw and an average body weight of 60 kg. For cured meats, up to 900 g could be consumed per day.

Consumption of these amounts of either food group on a daily basis is unlikely. The maximum amounts reported for cheese consumers were 99 g/day for the Australian population and 108 g/day for the New Zealand population at the 95th percentile of consumption, 62 g/day for the adult population of the United Kingdom at the 97.5th percentile of consumption, and 45 g/day for the population of the USA at the 90th percentile of consumption. Dietary records do not distinguish whether the cheese consumed had been cut, shredded, or grated but report all cheese consumed, either directly or indirectly in mixed foods.

The maximum amounts reported for consumption of comminuted meat were 170 g/day for the Australian population and 210 g/day for the New Zealand population, both at the 95th percentile of consumption.

3.2 Individual dietary records

The intake of natamycin estimated from individual dietary records was available for five countries (Table 7). The estimates were all well below the ADI, when either draft GSFA or national use levels were assumed.

Table 7. Intake of natamycin estimated from individual dietary records

Country and reference	Population group	Natamycin intake (mg/kg bw per day)	% ADI ^a	Assumptions	Survey	Date of survey
Australia (Australia–New Zealand Food Authority, 2001a)	All respondents	Mean, 0.017	5.6	GSFA permissions	National survey, single 24-h recall; sample, 13 858; age, ≥ 2 years; mean weight, 67 kg; calculations based on individual body weights	1995
	Consumers only	Mean, 0.026	8.5			
	Consumers only	Median, 0.016	5.5	Australia New Zealand Food Standards Code permissions: cheese, 15 mg/kg; salami, 1.2 mg/kg		
	Consumers only	95th percentile, 0.081	27			
New Zealand (Australia–New Zealand Food Authority, 2001b)	All respondents	Mean, 0.005	1.5	Australia New Zealand Food Standards Code permissions for cheese and salami	National survey; sample, 4636; single 24-h recall; age, ≥ 15 years; mean weight, 71 kg; calculations based on individual body weights	1997
	Consumers only	Mean, 0.009	3.0			
	Consumers only	Median, 0.006	2.0			
	Consumers only	95th percentile, 0.028	9.2			
New Zealand (Australia–New Zealand Food Authority, 2001b)	All respondents	Mean, 0.013	4.4	GSFA permissions	National survey; sample, 4636; single 24-h recall; age, ≥ 15 years; mean weight, 71 kg; calculations based on individual body weights	1997
	Consumers only	Mean, 0.022	7.3			
	Consumers only	Median, 0.015	5.1	Australia New Zealand Food Standards Code permissions for cheese and salami		
	Consumers only	95th percentile, 0.068	22			
United Kingdom (DSM Food Specialities, 2001)	All respondents	Mean, 0.003	1.1	Consumers represent 75% of the population; cheese only; GSFA level, 40 mg/kg ^a	National survey; 7-day record; adults 16–64 years; sample, 2197; assumed weight, 60 kg	1986–87
	Consumers only	Mean, 0.008	2.5			
	Consumers only	Median, 0.005	1.7			
	Consumers only	95th percentile, 0.021	7.1			
USA (DSM Food Specialities, 2001)	Adult consumers	Mean, 0.014	4.6	Consumers represent 46% of the population; cheese only.	Department of Agriculture Continuing Survey of Food Intakes; 2-day intake (one 24-h record plus one self-reported 1-day intake; weighted data; assumed weight, 60 kg)	1994
		90th percentile, 0.027	9.0			
		97.5th percentile, 0.041	14	GSFA level, 40 mg/kg ^a		
	Consumers only	Mean, 0.015	5.0			
	Consumers only	90th percentile, 0.032	11	Use level, 20 mg/kg		
	Consumers only	Mean, 0.007	2.3			
	Consumers only	90th percentile, 0.016	5.3			

Country and reference	Population group	Natamycin intake (mg/kg bw per day)	% ADI ^a	Assumptions	Survey	Date of survey
Germany (DSM Food Specialities, 2001)	Consumers only, 4–10 years	Mean, 0.015 90th percentile, 0.032 97.5th percentile, 0.051	5.0 11 17	Use level: cheese, 20 mg/kg; meats, 6 mg/kg (8.2.1.2) to 20 mg/kg (8.3.1.2)	National Food Intake Survey; sample, 15 838 (1359 4–10 years; 14 479 > 10 years)	1985–89 except April 1986–April 1987 ^b
	Consumers > 10 years	Mean, 0.01 90th percentile, 0.021 97.5th percentile, 0.031	3.3 7.0 10			

^a Intakes estimated from data given in submission from DSM Food Specialities (2001), assuming draft GSFA permissions

^b After Chernobyl accident

The estimates based on proposed GSFA levels of use from Australia and New Zealand were for cheese and meat sources. The mean intake for Australian consumers was estimated to be 0.026 mg/kg bw per day, or 9% of the ADI; that for New Zealand consumers was 0.022 mg/kg bw per day, or 7% of the ADI. Cheese contributed 72% of the total natamycin intake in Australia and 67% of that in New Zealand.

Estimates of the intake of natamycin in the United Kingdom and the USA were based on the proposed GSFA use in cheese. The mean estimated intakes were slightly lower than those reported for Australia and New Zealand: 0.014 mg/kg bw per day, or 5% of the ADI, for consumers in the United Kingdom and 0.015 mg/kg bw per day, or 5% of the ADI, for consumers in the USA. The intakes of consumers at high percentiles based on draft GSFA levels of use of natamycin ranged from 0.03 to 0.08 mg/kg bw per day (11–27% of the ADI).

National estimates of natamycin intake, submitted by Australia, Germany, New Zealand, and the USA, were all well below the ADI (Table 7), the mean intakes of consumers being 0.008–0.015 mg/kg bw per day (2.5–5% of the ADI), as the national permitted levels of use were much lower than those proposed in the draft GSFA, and use of natamycin was further restricted: 15 mg/kg in cheese and 1.2 mg/kg in salami in Australia and New Zealand; 20 mg/kg in cheese, 6 mg/kg in meats (8.2.1.2), and 20 mg/kg in meats (8.3.1.2) in Germany; and 20 mg/kg in cheese in the USA.

3.3 Evaluation of intake estimates

The submissions indicated that the intake of natamycin was well below the ADI and that the ADI was not likely to be exceeded even by consumers at high percentiles. The higher estimates for consumers at high percentiles in Australia and New Zealand (27% and 23% of the ADI, respectively) were due to use of single 24-h recall data, which tend to result in overestimates of the habitual intake of consumers at high percentiles. In the surveys of food consumption in the United Kingdom and the USA, the amounts were averaged over a number of days (3 and 7, respectively), which would tend to decrease the reported daily consumption of all foods and of occasionally consumed foods, such as salami type meats, in particular (Gibney, 1999; Lambe et al., 2000).

4. COMMENTS

Toxicological data

The Committee considered eight studies that had not been evaluated previously; these studies had been conducted more than 20 years earlier. A study of single intraperitoneal administration was considered to be irrelevant to the safety assessment of an ingested substance. The results of two studies of genotoxicity in three bacterial systems (*Bacillus subtilis*, *Salmonella typhimurium*, and *Escherichia coli*) were negative.

Two studies in rats and one in dogs given radiolabelled material for investigation of the distribution and elimination of the compound supported the previous conclusion that natamycin is excreted primarily in the faeces, with minimal absorption. The only adverse effect reported in a short-term study of toxicity in dogs was diarrhoea, which occurred most frequently in animals at the high dose (equivalent to 25 mg/kg bw per day); however, the usefulness of this study was limited as only two dogs were tested.

In a study of developmental toxicity, an aqueous suspension of 50% natamycin was given to groups of 20–26 mated rabbits at a dose of 0, 5, 15, or 50 mg/kg bw per day by gavage on days 6–18 of gestation. The maternal mortality rate was 0%, 5%, 9%, and 19% at the four doses, respectively. No clinical signs of toxicity were observed in the does, and the cause of death was unknown. Mean maternal body weight, pregnancy rate, number of implantation sites, number of resorption sites, numbers of live and dead fetuses, per cent viability, and incidence of soft-tissue anomalies were comparable in treated groups and a control group given the vehicle only. The fetal body weight at the intermediate dose was lower than that of the vehicle control group. The incidence of extra sternbrae was increased at the two higher doses in comparison with the vehicle control group, but not in a dose-related manner. However, in view of the known and unusual sensitivity of the gastrointestinal tract of rabbits to poorly absorbed substances and to compounds with antimicrobial activity, this study was not considered suitable for deriving the ADI.

Microbiological data

The antifungal activities of natamycin and other polyenes depend on their binding to cell membrane sterols, primarily ergosterol, the principal sterol in fungal membranes. Oomycete fungi and bacteria are insensitive to these antibiotics because their membranes lack ergosterol.

Use of natamycin as an antifungal agent in food may result in exposure of the endogenous microflora to trace quantities of antimicrobial residues. The human intestinal microflora are a complex mixture of more than 400 bacterial species, composed primarily of bacterial cells at a concentration of 10^{11} – 10^{12} colony forming units per gram. Fungi are much less abundant in the human gastrointestinal tract than bacteria, up to 10^5 colony forming units per gram of yeast being reported in stool samples from healthy subjects. As bacteria are not affected by polyenes, natamycin residues should not harm them, and as yeasts are found in low quantities the consequences of exposure to traces of natamycin would be minimal.

Several studies in experimental animals indicated a lack of antibiotic activity in the colon, suggesting that natamycin was degraded into microbiologically inactive compounds by bacterial flora. However, no data are available on the degradation of natamycin by human intestinal microflora. In one study, natamycin was present in faecal specimens of volunteers who ingested 500 mg of the compound, indicating that the compound is incompletely absorbed or degraded.

As emergence of antibiotic resistance is a concern, the Committee evaluated the possible development of resistance among microflora as a consequence of ingestion of natamycin. A 50% natamycin preparation has been used for over 20 years to preserve cheese and sausages. Surveys in cheese warehouses and in dry-sausage factories where the preparation has been used showed no change in the composition or the sensitivity of the contaminating fungal flora. All but one of the species of yeasts and moulds isolated in cheese warehouses where natamycin was used were inhibited at the same low concentrations (0.5–8 µg/ml). In another study, 26 fungi were isolated in eight warehouses where natamycin was used and two warehouses where it never had been used and tested for sensitivity to the compound; no insensitive yeasts or moulds were found. The results of laboratory experiments intended to induce resistance to natamycin in strains isolated from cheese warehouses indicated that, after 25–30 transfers to media with increasing concentrations of natamycin, none of the strains had become less sensitive. When the sensitivity of yeasts and moulds isolated from dry-sausage factories where natamycin had been used for several years was compared with that of isolates from factories where natamycin had never been applied, no significant differences were demonstrated.

Induction of polyene-resistant and especially natamycin-resistant mutants *in vitro* is difficult. Such mutants invariably show reduced metabolic and growth rates and, in the absence of polyenes, readily revert to normal metabolism, growth, and sensitivity to natamycin. One means of obtaining resistant isolates is successive sub-culturing *in vitro* in the presence of gradually increasing concentrations of the polyene. Typically, such isolates are resistant only up to the highest concentration to which they have been exposed. After 25 passages, the microbiological inhibitory concentration of *Candida albicans* was minimally increased from 2.5–12 to 12–50 µg/ml.

Assessment of intake

Application of the budget method indicated that further assessment of the intake of natamycin was required. The draft GSFA proposes restricted use of natamycin in cheese (category 1.6) and dried, non-heat-treated meat groups (categories 8.2.1.2 and 8.3.1.2) only, so that the intake would not be expected to exceed the ADI.

Submissions from Australia, Germany, New Zealand, the United Kingdom, and the USA indicated that the intakes at the mean and high levels of consumption were well below the ADI, although the estimates for the United Kingdom and the USA covered cheese consumption only. The estimated mean intakes for consumers ranged from 0.01 to 0.03 mg/kg bw per day (representing 3 and 9% of the ADI in Germany and the United Kingdom, respectively), and those for high consumers were 0.03–0.08 mg/kg bw per day (representing 9 and 27% of the ADI in Australia and the United Kingdom, respectively), if it is assumed that natamycin was used at 40 mg/kg in all cheese products and 20 mg/kg in all cured meat products, as proposed in the draft GSFA. The estimated intakes of natamycin were lower when national use levels were assumed.

5. EVALUATION

Although use of natamycin as an antifungal agent in food may result in exposure of the endogenous flora to trace quantities of antimicrobial residues, bacteria in the human gastrointestinal tract are not affected by polyenes, and the Committee concluded that disruption of the colonization barrier is not a concern. Fungi are found in much smaller amounts than bacteria in the human gastrointestinal tract, and the negative results in studies of acquired resistance indicate that selection of natamycin-resistant fungi is not an issue.

The Committee noted the finding of extra sternebrae in the study of developmental toxicity in rabbits, in which a dose-related increase in the mortality rate was reported. It considered that administration of an antimicrobial agent to rabbits by gavage was inappropriate. In addition, extra sternebrae have been described as a skeletal variation rather than a frank indication of teratogenicity. Thus, the Committee did not consider this result to be evidence that natamycin is teratogenic.

The Committee confirmed the previously established ADI of 0–0.3 mg/kg bw for natamycin, which was based on observations of gastrointestinal effects in humans. The Committee noted that the estimated intake of natamycin based on maximum levels of use in cheese and processed meats proposed in the draft GSFA does not exceed this ADI.

6. REFERENCES JECFA, 2002

Anonymous (1965) Data on the safety of the use of pimaricin as preservative against mold growth on cheese. Summary of the results of acute and chronic toxicity tests. Unpublished report submitted to WHO by the Royal Netherlands Fermentation Industries Ltd, Delft.

Anonymous (1968) Absorption of pimaricin following oral administration. Unpublished report submitted to WHO by the Royal Netherlands Fermentation Industries Ltd, Delft.

Aparicio, J.F., Colina, A.J., Ceballos, E. & Martin, J.F. (1999) The biosynthetic gene cluster for the 26-membered ring polyene macrolide pimaricin. *J. Biochem. Mol. Biol.*, **274**, 10133–10139.

Athar, M.A. & Winner, H. I. (1971) The development of resistance by *Candida* species to polyene antibiotics in vitro. *J. Med. Microbiol.*, **4**, 505–517.

Australia New Zealand Food Authority (2001a) Submission on estimated pimaricin intakes to WHO on behalf of the Australian Government.

Australia New Zealand Food Authority (2001b) Submission on estimated pimaricin intakes to WHO on behalf of the New Zealand Government.

Bailey, D.E. & Morgareidge, K. (1974) Teratogenicity test with pimaricin. Unpublished report No. 1-1052 submitted to WHO by Food and Drug Research Laboratories Inc.

Bernhardt, H. (1998) Fungi in the intestine—Normal flora or pathogens? *Z. Arztliche Fortbild. Qual.*, **92**, 154–156.

Blankwater, Y.J. & Hespe, W. (1979) Autoradiographic and bioautographic study of the distribution of oral natamycin in the rat. Unpublished report No. 20.502, dated 8 May 1979 from Gist-Brocades NV, Delft.

Blaschke-Hellmessen, R., Buchmann, H. & Schwarze, R. (1996) Effect of orally administered polyene antimycotics on the intestinal colonization with yeasts: Possibilities and limitations. *Mycoses*, **39**, 33–39.

de Boer, E. & Stolk-Horsthuis, M. (1977) Sensitivity to natamycin (pimaricin) of fungi isolated in cheese warehouses. *J. Food Prot.*, **40**, 533–536.

de Boer, E., Labots H., Stolk-Horsthuis, M. & Visser, J.N. (1979) Sensitivity to natamycin of fungi in factories producing dry sausage, *Fleischwirtschaft*, **59**, 1868–1869.

Bodenhoff, J. (1968) [Resistance of *Candida albicans*, with besonderer Berücksichtigung von zwei während einer längeren Zeit mittels antibiotica behandelten Patienten.] *Scand. Dent. J.*, **76**, 279 (in German).

- Brik, H. (1975) Natamycin (pimaricin). New high-molecular decomposition products with intact lactone-ring. Unpublished report submitted to WHO by Gist-Brocades NV, Delft.
- Brik, H. (1981) Natamycin. *Anal. Profiles Drug Substances*, **10**, 513–561.
- Carlile, M.J. & Watkinson, S.C., eds (1994) Fungal cells and vegetative growth. In: *The Fungi*, San Diego, CA: Academic Press, p. 148.
- Cerniglia, C.E. & Kotarski, S. 1999. Evaluation of veterinary drug residues in food for their potential to affect human intestinal microflora. *J. Regul. Toxicol. Pharmacol.*, **29**, 238–261.
- Cox, G.E., Bailey, D.E. & Morgareidge, K. (1973) Unpublished report No. 1-1052 submitted to WHO by Food and Drug Research Laboratories Inc.
- Deacon, J.W., ed. (1997) Prevention and control of fungal growth. In: *Modern Mycology*, 3rd Ed., Oxford: Blackwell Science, pp. 289–290.
- Dekker, J. & Gielink, A.J. (1979) Acquired resistance to pimaricin in *Cladosporium cucumerinum* and *Fusarium oxysporum* f.sp. *narcissi* associated with decreased virulence. *Neth. J. Plant Pathol.*, **85**, 67–73.
- DSM Food Specialities (2001) Submission on pimaricin to WHO from DSM Food Specialities USA, Inc., King of Prussia, Pennsylvania, USA.
- van Eeken, C.J. & Wubs, W. (1976) Acute intraperitoneal toxicity of natamycin and three potential metabolites. Unpublished report No. 15.465, 11 January 1976, submitted to WHO by Gist-Brocades.
- van Eeken, C.J., Birtwhistle R.D.R. & Aboulwafa-wan Velthoven, M.J.E. (1984) Three months study in dogs of the toxicity of natamycin by addition to the food. Unpublished report No. 12.401, 24 October 1984, submitted to WHO by Gist-Brocades Research and Development.
- Euichi, H., Kamada, Y., Kanagawa, T., Naito, T. & Shiota, H. (1999) Cases of keratomycosis due to *Fusarium* species. *Rinsho Ganka (Jpn. J. Clin. Ophthalmol.)*, **53**, 609–611.
- Farkas, J. & Kiss, I. (1976) The effect of pimaricin on the microbial flora of cottage cheese in plastic packaging. *Acta Aliment.*, **5**, 107–118.
- Food and Drug Administration (2001) Food additives permitted for direct addition to food for human consumption: Natamycin (pimaricin). Final rule. *Fed. Reg.*, **66**, 21 CFR Part 172.
- Franklin, T.J. & Snow, G.A., eds (1998) Antiseptics, antibiotics and the cell membrane. In: *Biochemistry and Molecular Biology of Antimicrobial Drug Action*, 5th Ed. Dordrecht: Kluwer Academic Publishers, pp. 55–56.
- Gibney, M.J. (1999) Dietary intake methods for estimating food additive intake. *Regul. Toxicol. Pharmacol.*, **30**, S31–S33.

- Griffin, D.A., ed. (1994) Fungicides. In: *Fungal Physiology*, 2nd Ed., New York: Wiley-Liss, Inc., pp. 416–417.
- Grupper, C. (1961) Personal communication from the Hôpital Saint-Louis, Paris.
- Grupper, C. (1964) Pimaricin in the treatment of superficial mucocutaneous monoliasis. In: Proceedings of the International Congress on Tropical Dermatoses, Naples, June 1964.
- Hamilton-Miller, J.M.T. (1974) Fungal sterols and the mode of action of the polyene antibiotics. In: Perlman, D., ed., *Advances in Applied Microbiology*, New York: Academic Press, pp. 109–134.
- Hejzlar, M. & Vymola, F. (1970) Comparative study of pimaricin and fungicidin activity *in vitro*. *J. Hyg. Epidemiol. (Praha)*, **14**, 211–213.
- Hespe, W. & Meier, A.M. (1980) Studies involving dogs in regard to the resorption of radioactivity following the oral administration of ¹⁴C-pimaricin, applied on cheese, in comparison to other oral forms of administration. Unpublished report No. 20.531, dated 4 February 1980, submitted to WHO by Gist-Brocades NV, Haarlem.
- Hoekstra, E.S. & Van der Horst, M.I. (1998) Survey of the fungal flora in Dutch cheese factories and warehouses. *J. Food Mycol.*, **1**, 13–22.
- Holly, R.A. (1981) Prevention of surface mold growth on Italian dry sausage by natamycin and potassium sorbate. *Appl. Microbiol.*, **41**, 422–429.
- Holly, R.A. (1986) Effect of sorbate and pimaricin on surface mold and ripening of Italian dry salami. *Lebensm.-Wiss. Technol.*, **19**, 59–65.
- Hutchison, E.B., Ribelin, W.E. & Levinskas, G.J. (1966) 98-day study in the rat. Unpublished report submitted to WHO by American Cyanamid Co.
- Jay, J.M. ed. (1996) *Modern Food Microbiology*, 5th Ed., New York: Chapman & Hall, pp. 293–294.
- Khoudokormoff, B. (1977) Short term microbial tests on mutagenicity of pimaricin (natamycin) and its products of degradation. Unpublished preliminary results, archive No. 10.545, 29 June 1977, submitted to WHO by Gist-Brocades NV.
- Khoudokormoff, B. (1978) Potential carcinogenicity of some food preservatives in the presence of traces of nitrite. *Mutat. Res.*, **53**, 208–209.
- Khoudokormoff, B. (1984) Are resistance development and morphological changes possible after use of natamycin? *Wein-Wiss.*, **39**, 45–50.
- Khoudokormoff, B. & Petru, M. (1974) On the possible development of antibiotic resistance amongst fungi with special reference to the use of pimaricin as a preservative in the food industry. Unpublished report submitted to WHO by Gist-Brocades NV, Delft.

- Knickerbocker, M. & Re, T.A. (1978) Teratologic evaluation of pimaricin in Dutch belted rabbits. Unpublished report from Food and Research Laboratories, Inc., Waverly Research Center, NY). FDRL Report No. 5906, 22 November 1978. Submitted to WHO by Gist-Brocades, Delft.
- Knickerbocker, M. & Re, T.A. (1979) Teratologic evaluation of pimaricin in Dutch belted rabbits (amendment). Unpublished report from Food and Research Laboratories, Inc., Waverly Research Center, NY). FDRL Report No. 5906, 26 April 1979. Submitted to WHO by Gist-Brocades, Delft.
- Kreisel, W. (1999) Fungi in the intestine. Clinical significance. *Schweiz. Rundsch. Med. Prax.*, **88**, 5–10.
- Lambe, J., Kearney, J., Leclercq, C., Berardi, D., Zunft, H.F.J., De Henauw, S., De Volder, M., Lamberg-Allardt, C.J.E., Karkkainen, M.U.M., Dunne, A. & Gibney, N.J. (2000) Enhancing the capacity of food consumption surveys of short duration to estimate long term consumer-only intakes by combination with a qualitative food frequency questionnaire. *Food Addit. Contamin.*, **17**, 177–187.
- Lavingia, B. & Dave, D. (1986) Comparative study of amphotericin-B, pimaricin and gentian violet on ocular fungi. *Indian J. Ophthalmol.*, **34**, 73–77.
- Levinskas, G.J., Shaffer, C.B., Bushey, C., Kinde, M.L., Stackhouse, D.W. & Vidone, L.B. (1963) Two-year feeding to rats. Unpublished report from the Central Medical Department, American Cyanamid Co. Submitted to WHO by
- Levinskas, G.J., Ribelin, W.E. & Shaffer, C.B. (1966) Acute and chronic toxicity of pimaricin. *Toxicol. Appl. Pharmacol.*, **8**, 97–109.
- Littman, M.L., Pisano, M.A. & Lancaster, R.M. (1958) Induced resistance of *Candida* species to nystatin and amphotericin B. In: *Antibiotics Ann.* 981. New York: Medical Encyclopedia.
- Lovgren, T. & Salmela, I. (1978) *In vitro* sensitivity of *Trichomonas vaginalis* and *Candida albicans* to chemotherapeutic agents. *Acta Pathol. Microbiol. Scand. Sect. B*, **80**, 155–158.
- Lüock, H. & Cheesman, C.E. (1978) Mould growth on cheese as influenced by pimaricin or sorbate treatments. *S. Afr. J. Dairy Technol.*, **10**, 143–146.
- Macura, A.B. (1991) Fungal resistance to antimycotic drugs. A growing problem. *Int. J. Dermatol.*, **30**, 131–183.
- Majewski, S. & Macua, A.B. (1978) Efficacy of topical antifungal treatment in denture stomatitis. *Mykosen*, **21**, 403–406.
- Malten, K.E. (1967) Report of an investigation concerning possible allergic side effects of pimaricin in humans. Unpublished report from the Instituut voor Geneeskunde en Maatschappij, Nijmegen. Submitted to WHO by Gist-Brocades NV, Delft.

- Malten, K.E. (1968) Report on investigation into possible sensitising side effects of pimaricin in human beings. Unpublished report from the Instituut voor Geneeskunde en Maatschappij, Nijmegen. Submitted to WHO by Gist-Brocades NV, Delft.
- Manson, J.M. & Kang, Y.J. (1994) In: Hayes, J.W., ed., *Principles and Methods of Toxicology*, 3rd Ed., New York: Raven Press, p. 1003.
- Masterton, G., Sengupta, S.M. & Schofield, C.B.S. (1975) Natamycin in genital candidosis in men. *Br. J. Vener. Dis.*, **51**, 210–212.
- McGinnis, M.R. & Rinaldi, M.G. (1985) Antifungal drugs: Mechanisms of action, drug resistance, susceptibility testing, and assays of activity in biological fluids. In: Lorian, V., ed., *Antibiotics in Laboratory Medicine*, 2nd Ed., Baltimore: Williams & Wilkins, pp. 223–281.
- Meier A.M. & Hesper, W. (1979). The metabolism of pimaricin in rats. II. Investigation, with the help of ¹⁴C-pimaricin, of its resorption, its decomposition in the gastrointestinal tract, and its elimination. Unpublished report 20.504, 28 May 1979.
- Mendes, M.V., Aparicio, J.F. & Martin, J.F. (1999) Complete nucleotide sequence and characterization of pSNA1 from pimaricin-producing *Streptomyces natalensis* that replicated by a rolling circle mechanism. *Plasmid*, **43**, 159–165.
- Molzahn, S.W. & Woods, R.A. (1972) Polyene resistance and the isolation of sterol mutants in *Saccharomyces cerevisiae*. *J. Gen. Microbiol.*, **72**, 339–348.
- Morgenstern, A.P. & Muskens, G.J.A.M. (1975) Further data on the toxicity of the decomposition products of pimaricin. Unpublished report submitted to WHO by Gist-Brocades NV, Delft
- Morris, H.A. & Castberg, H.B. (1980) Control of surface growth on blue cheese using pimaricin. *Cult. Dairy Prod. J.*, **15**, 21–23.
- Mukhtar, H., Hakkou, A. & Bonaly, R. (1994) Studies on the activity of *Kluyveromyces lactis* S-adenosylmethionine: Δ 24-sterol methyltransferase in presence of polyenic antifungal agents. *Mycopathologia*, **126**, 75–83.
- Newcomer, V.D., Sternberg, T.H., Wright, E.T., Reisner, R.M., McNall, E.G. & Sorensin, L.J. (1960) The treatment of systemic diseases with orally administered pimaricin: Preliminary report. *Ann. N.Y. Acad. Sci.*, **89**, 240–246.
- Nilson, K.M., Shahani, K.M., Vakil, J.R. & Kilara, A. (1974) Pimaricin and mycostatin for retarding cottage cheese spoilage. *J. Dairy Sci.*, **58**, 668–671.
- Norman, A.W., Spielvogel, A.M. & Wong, R.G. (1976) Polyene antibiotic-sterol interaction. *Adv. Lipid Res.*, **14**, 127–170.

- Novak, E.K., Barbarics, E., Vincze, I. & Zala, J. (1984) *In vitro* studies on the food preservative antifungal polyene antibiotic pimaricin (natamycin). In: Kiss, I., Deck, T. & Incaze, K., eds, *Microbial Association and Interactions in Food*, Dordrecht: Kluwer..
- O'Day, D.M., Ray, W.A., Robinson, R.D., Head, W.S. & Savage, A.M. (1987) *In vitro* and *in vivo* susceptibility of *Candida* keratitis to topical polyenes. *Invest. Ophthalmol. Visual Sci.*, **28**, 874–880.
- Oldenkamp, E.P. (1979) Natamycin treatment of ringworm in cattle in the United Kingdom. *Vet. Rec.*, **105**, 554–556.
- Oldenkamp, E.P. & Spanoghe, L. (1977) Natamycin-S treatment of ringworm in cattle. *Tijdschr. Diergeneeskd.*, **102**, 124–125.
- Ottens, H. (1965) Unpublished report submitted to WHO by Royal Netherlands Fermentation Industries, Delft.
- Pedersen, J.C. (1992) Natamycin as a fungicide in agar media. *Appl. Environ. Microbiol.*, **58**, 1064–1066.
- Pugazhenthii, T.R, Dhanalakshmi, B., Narasimhan, R., Shibu, A.V. & Madhan, S. (1999) Effect of anti-mycotic agents on *Penicillium citrinum* in cheese. *Indian Vet. J.*, **76**, 537–539.
- Raab, W. P. (1972) *Natamycin (Pimaricin). Its Properties and Possibilities in Medicine*, Stuttgart: Georg Thieme Publishers.
- Reynolds, J.E.F., ed. (1996) *Martindale. The Extra Pharmacopoeia*, 31st Ed., London: Pharmaceutical Press.
- Rusul, G. & Marth, E.H. (1988) Growth and aflatoxin production by *Aspergillus parasiticus* in a medium at different pH values and with or without pimaricin. *Z. Lebensm. Unters. Forsch.*, **187**, 436–439.
- Sacjdeva, S. & Singh, S. (1985) Pimaricin—A potential preservative in the cheese industry. *Indian Dairyman*, **37**, 587–596.
- Safe, L.M., Safe, S.H., Subden, R.E. & Morris, D.C. (1977) Sterol content and polyene antibiotic resistance in isolates of *Candida krusei*, *Candida parakrusei*, and *Candida tropicalis*. *Can. J. Microbiol.*, **23**, 398–401.
- Scheurlen, M. (1996) Pathogenicity of fungi in the intestines—Current status of the discussion. *Fortschr. Med.*, **114**, 319–321.
- Shahani, K.M., Bullerman, L.B., Evans, T.A. & Arnold, R.G. (1977) Prevention of toxin mold growth in cheese by pimaricin. *Arch. Inst. Pasteur Tunis*, **43**, 511–520.

- Sörensen, L.J., McNall, E.G. & Sternberg, T.H. (1959) The development of strains of *Candida albicans* and *Coccidioides immitis* which are resistant to amphotericin B. In: *Antibiotics Annual 1958–1959*, New York: Medical Encyclopedia, pp. 920–923.
- Stern, G.A. (1978) *In vitro* antibiotic synergism against ocular fungal isolates. *Am. J. Ophthalmol.*, **86**, 359–367.
- Stout, H.A. & Pagano, J.F. (1956) Resistance studies with nystatin. In: *Antibiotics Ann.*: 704. New York
- Struyk, A.P., Hoette, I., Drost, G., Waisvisz, J.M., van Eek, T. & Hoogerheide, J.C. (1958) Pimaricin, a new antifungal antibiotic. In: Welch, H. & Marti-Ibanez, F., eds, *Antibiotics Annual 1957–1958*, New York: Medical Encyclopedia, Inc., pp. 878–885.
- Subden, R.E., Safe, L., Morris, D.C., Brown, R.G., and Safe S. (1977) Eburicol, lichesterol, ergosterol, and obtusifoliol from polyene antibiotic-resistant mutants of *Candida albicans*. *Can. J. Microbiol.*, **23**, 751–754.
- Walter, A.M. & Heilmeyer, L. (1969) *Antibiotika Fibel*. Stuttgart: Thieme Verlag.
- Wieriks, J. (1966) Pimaricin in cheese: A toxicity test of seven weeks in rats. Unpublished report from the Royal Netherlands Fermentation Industries Ltd, submitted to WHO.
- Wieriks, J. (1971) Pimaricin in apples: A toxicity test of three months in rats. Unpublished report from the Royal Netherlands Fermentation Industries Ltd, submitted to WHO.

Annex IX-2

ETA position on food allergen labeling of microbially derived enzymes under FALCPA as it applies to fermentation media raw materials



ENZYME TECHNICAL ASSOCIATION

1800 Massachusetts Avenue, NW, 2nd Floor
Washington, DC 20036-1800

Telephone (202) 778-9335
Fax (202) 778-9100
www.enzymetechnicalassoc.org

POSITION PAPER

ETA Position On Food Allergen Labeling of Microbially Derived Enzymes Under FALCPA as it Applies to Fermentation Media Raw Materials

It is the position of the Enzyme Technical Association (ETA) that microbially derived enzymes do not fall within the scope of the Food Allergy Labeling and Consumer Protection Act (FALCPA) and that labeling for food allergens is not triggered by the use of a microbially derived enzyme preparation. There may be other reasons why a manufacturer labels a food product with regard to allergen content, but the use of a microbially derived enzyme preparation is not a reason for such labeling.

Enzymes are not one of the eight major allergenic foods, often referred to as the big 8, so they do not fit within the first requirement of FALCPA. In addition, microbial enzymes are not byproducts of nor are they derived from the major food allergens. Although enzymes are not major food allergens,¹ many enzymes are produced with microorganisms and the nutrient media used to feed these microorganisms may contain protein from one or more of the major food allergens. The enzymes are not derived from raw materials containing major food allergens, but rather are obtained from the microorganisms which are used to produce the enzyme proteins. In other words, enzymes obtained from fermentation are directly derived from microorganisms fed on media that may include protein obtained from one or more of the major food allergens. Proteins and other nitrogenous material are consumed by the microorganisms for cell growth, cell maintenance, and production of enzyme protein. It is the intent of the enzyme manufacturer to supply enzymes, therefore it is critical that the ratio of nutrient to enzyme yield is carefully controlled. It is also the intent of the manufacturer that these raw materials are added to the fermentation as food to be consumed by the microorganism and are not added as formulation ingredients.

In arriving at its position ETA also considered that:

- The regulatory agencies in the EU and Japan have determined that enzyme preparations are not required to have allergen labeling for the raw materials used in the fermentation process. Indeed, the European Commission's Health & Consumer Protection Directorate General has clearly stated that enzymes

¹ To the extent the enzyme producer uses an allergenic material, such as wheat flour diluent in the final product formulation, labeling may be required.

are outside the scope of the Directive 2003/89/EC which amended the EU Food Labelling Regulations.

- Enzyme broths are normally processed to separate biomass and fermentation materials from the enzyme, to concentrate the enzymatic activity, and formulated to achieve a uniform and stable enzyme product.
- The unique role of enzymes in food processing is as a catalyst. Due to the specific nature of enzymes, only small amounts are required to make desired modifications to the property of a food.
- Many enzymes do not become a component of the food ingredient or final food. Some enzymes are used in an immobilized form or are denatured during processing. Further, processing of the food ingredient after the enzyme catalyst has performed the expected function often reduces or eliminates the enzyme from the product.
- ETA has made an extensive review of the published scientific literature and has found no reports that even suggest there has been an allergic reaction to a component of the fermentation media which was used to feed the microorganism that produced the enzyme.

The above position paper and accompanying report were provided to FDA on September 12, 2005 and to date ETA has received no comment.



Annex IX-3

Expert opinion statement Food Allergy Research & Resource Program
University of Nebraska

Testing of microbially derived enzymes for potential allergens from fermentation media
raw materials

EXPERT OPINION STATEMENT
FOOD ALLERGY RESEARCH & RESOURCE PROGRAM
UNIVERSITY OF NEBRASKA

**Testing of Microbially Derived Enzymes for Potential Allergens from
Fermentation Media Raw Materials**

August 13, 2013

Prepared by: Steve L. Taylor, Ph.D., Co-Director
and
Joe L. Baumert, Ph.D., Co-Director

with assistance from Enzyme Technical Association

Microbially derived enzymes are used by food processors as additives and processing aids in a wide variety of foods. Enzymes obtained from microbial fermentation are directly derived from microorganisms fed on sterilized media¹ that may include protein sources obtained from one or more of the recognized commonly allergenic foods (e.g., milk, soybean) or from a cereal source of gluten (e.g., wheat, barley). This paper addresses the relevance of testing microbial enzymes for allergenic material from the fermentation growth media.²

It has been the long-standing position of the Food Allergy Research & Resource Program (FARRP) at the University of Nebraska that testing of the products of fermentation (with limited exceptions), including microbially derived enzymes is unreliable using enzyme-linked immunosorbent assays (ELISAs).

While various fermentation media may contain one or more of the major food allergens, the biochemical reactions that occur during fermentation result in the breakdown of the fermentation media proteins. The extent of proteolysis is dependent upon the fermentation culture and the resultant enzyme (e.g., some enzymes are proteases). As proteins are digested, the resulting amino acids, along with other nitrogenous material, are consumed by the microorganisms for cell growth, cell maintenance, and production of enzyme protein.

¹ Aunstrup, K., O. Andresen, E.A. Falch, and T.K. Nielsen (1979) *Microbial Technology*. (Perlman and Pepler, eds.) Academic Press, pp. 281-309.

² For this paper, FARRP's analysis is limited to microbially derived enzymes that are intended for additive and processing aid applications in food.

Upon completion of fermentation, remaining fermentation media that are not consumed by the microorganism are typically separated and/or purified from the enzyme in the recovery process. Enzymes are recovered from the fermentation broth by standard chemical engineering operations, such as filtration and centrifugation, broadly used in enzyme production.^{3,4} (See Appendices for further information.) The recovery steps result in separation of microbial biomass and other fermentation solids from the enzyme, concentration of the enzyme, and removal of impurities prior to final formulation with food-grade ingredients.

Any potential residual fragments from the food allergen would be difficult to measure as there is no reliable assay. Commercial ELISAs are able to detect only intact proteins in most cases. Any peptides, even larger ones, would not likely be detected, although this possibility has not been well investigated. Results would typically be reported as below the limit of quantitation for the enzyme preparation. Further, if any residual but undetected fragments of the food allergen remain, the relevance of any such residual material to food allergenicity is unproven. Accordingly, testing of fermented product does not result in reliable or useful data.

In addition, due to the specific catalytic nature of enzymes, only very small amounts of enzymes are generally required and used by food processors to make the desired modifications to the property of a food, and therefore any *de minimis* amount of fermentation media protein that may survive the fermentation process will not pose a significant public health risk to the consumer.⁵

FARRP also notes that regulatory agencies in the European Union and Japan do not require allergen labeling of enzyme preparations for the raw materials used in the fermentation process.

³ Atkinson, B. and F. Mavituna (1991) *Biochemical Engineering and Biotechnology Handbook*. (Atkinson, B. and Mavituna, F., eds.) Stockton Press, Hampshire, pp. 1146-1158.

⁴ Kroschwitz, J.I. (1994) *Enzyme Applications in Encyclopedia of Chemical Technology*. 4th edition, Volume 9. (Kroschwitz, J.I., ed.), pp. 567-620.

⁵ To the extent the enzyme producer uses an allergen as diluent to formulate the final product, labeling for such allergen is appropriate and required under Food Allergen Labeling and Consumer Protection Act.



THIS PAGE IS LEFT BLANK INTENTIONALLY

References related to this GRAS Notice

Anonymous (1968), Absorption of pimaricin following oral administration, Unpublished internal DSM report, submitted to WHO/JECFA by DSM.

Bailey D.E. & Morgareidge K. (1974), Teratogenicity test with pimaricin. Unpublished DSM report No. 1-1052, commissioned at Food and Drug Research Laboratories Inc.; submitted to WHO/JECFA by DSM

Bailey D. E. & Morgareidge K. (1974), Mutagenic studies in Rats (Including teratologic evaluation in Rabbits) with Delvocid brand of Pimaricin, Addendum II to Reports No. 9268 and 9359. Unpublished DSM report No. 1-1052, commissioned at Food and Drug Research Laboratories Inc.; submitted to WHO/JECFA by DSM

Blankwater Y.J. & Hesper W. (1979), Autoradiographic and bioautographic study of the distribution of oral natamycin in the rat. Unpublished internal DSM report No. 20.502, dated 8 May 1979, submitted to WHO/JECFA by DSM

Boer de E. & Stolk-Horsthuis M. (1977), Sensitivity to natamycin (Pimaricin) of fungi isolated in cheese warehouses, *J. Food Protection* 40:533

Boor K., Fromm H. (2006), Managing microbial spoilage in the dairy industry in *Food spoilage microorganisms*, C. de W. Blackburn (ed.), Woodhead Publishing Ltd

Brik H. (1976), Natamycin (pimaricin). New high-molecular decomposition products with intact lactone-ring, *Journal Antibiotics* 29:632-637

Brik H. (1981), Natamycin (review article); *Analytical profiles of drug substances* 10:513-561

Brik H. (1994), Natamycin (Supplement), *Analytical Profiles of Drug Substances*, 23: 399 - 419

Bullerman L.B. and Olivigni J. (1974), Mycotoxin producing potential of moulds isolated from Cheddar cheese. *J. Food Science* 39: 1166

Bullerman L.B. (1976), Examination of Swiss cheese for incidence of mycotoxin producing molds, *Journal of Food Science* 41: 26-28

Bullerman L.B. (1977), Incidence and control of mycotoxin producing molds in domestic and imported cheeses, *Annales de la Nutrition et de l'Alimentation* 31: 435-446

Cox G.E., Bailey D.E. and Morgareidge K. (1973), Multigeneration reproduction studies in rats with Delvocid brand of pimaricin (including teratologic and mutagenic phases), unpublished DSM report No. 1-1052, commissioned at Food and Drug Research Laboratories Inc.; submitted to WHO/JECFA by DSM

Crutchfield V. (2008), Acute inhalation toxicity study in rats. Unpublished DSM report, commissioned at Stillmeadow Inc. Study number 11405-07

De Ruig W.G., Van der Berg G. (1985), Influence of the fungicides sorbate and natamycin in cheese coatings on the quality of the cheese, Netherlands Milk and Dairy Journal, 39, 165-172

Dekker A. (2012), Effect of sorbate and Natamycin on US yogurt starter cultures and yeast inhibition. Unpublished internal DFS study (#00058521), March 26, 2012

Ellin Doyle M. (2007), Microbial food spoilage - Losses and control strategies A brief review of the literature, Food Research Institute, University of Wisconsin-Madison
Available at: http://fri.wisc.edu/docs/pdf/FRI_Brief_Microbial_Food_Spoilage_7_07.pdf

El-Diasty, Eman M., El-Kaseh R.M. and Salem R.M., (2008)
The effect of natamycin on keeping quality and organoleptic characters of yoghurt
Arab J. Biotech., Vol. 12, No. (1): 41-48

EFSA, 2009, Scientific Opinion on the use of natamycin (E 235) as a food additive,
Available at: <http://www.efsa.europa.eu/en/efsajournal/doc/1412.pdf>

Exponent, Inc., (2013) Estimated daily intake of natamycin from proposed uses in yogurt products, Report 1304507.000-2824

Fente-Sampayo C.A., Vazquez-Belda B., Franco-Abuin C. et al. (1995), Distribution of fungal genera in cheese and dairies. Sensitivity to potassium sorbate and natamycin. Archiv für Lebensmittelhygiene, 46 (3): 62-65.

Fleet G.H., (1990), A review Yeast in dairy products
Journal of Applied Bacteriology, 68, 199 - 211

Food and Drug Administration (1998) Food additives permitted for direct addition to food for human consumption: Natamycin (pimaricin), Final rule, Fed. Reg., Vol 63, No 230, 66015

Food and Drug Administration (2004) Food Additives Permitted in Feed and Drinking Water of Animals; Natamycin, Final rule, Fed. Reg., Vol 69, No 71, 19320

Grupper C. (1961), Personal communication from the Hôpital Saint-Louis, Paris

Hamilton-Miller J.M. (1973), Chemistry and biology of the polyene macrolide antibiotics, Bacteriol. Rev. 1973, 37(3):166

Hespe W., Meier A.M. (1980), Studies involving dogs in regard to the resorption of radioactivity following the oral administration of ¹⁴C-pimaricin, applied on cheese, in comparison to other oral forms of administration. Unpublished internal DSM report No. 20.531, dated 4 February 1980, submitted to WHO/JECFA by DSM

Hoekstra E.S., van der Horst M.I., Samson R.A., Stark J., van Rijn F.T.J. (1998), Survey of the fungal flora in Dutch cheese factories and warehouses. *J. of Food Mycology* 1(1):13-22

Holley R.A. (1981), Prevention of surface mold growth on Italian dry sausage by natamycin and potassium sorbate, *Appl. Environ. Microbiol.* 1981, 41(2):422

JECFA, (2002), Natamycin. Evaluation of certain food additives, Report of the Joint FAO/WHO Expert Committee on Food Additives (57th meeting), WHO Food Additives Series 48, pp. 49-76

Available at: <http://www.inchem.org/documents/jecfa/jecmono/v48je06.htm>

Khoudokormoff B. & Petru M. (1974), On the possible development of antibiotic resistance amongst fungi with special reference to the use of pimaricin as a preservative in the food industry. Unpublished internal DSM report, submitted to the WHO/JECFA by DSM

Khoudokormoff B. (1977), Potential carcinogenicity of some food preservatives in the presence of traces of nitrite, *Mutation Research* 53:208-209

Khoudokormoff B. (1983), Short-term microbial tests on mutagenicity of natamycin and its products of degradation. Unpublished internal DSM report nr 10.545

Klis J.B. and Witter L.D. (1959), The effect of several antifungal antibiotics on the growth of common food spoilage fungi, *Food Technol.* 13:124-128

Koninklijke Nederlandsche Gist- en Spiritus-Fabriek N.V. (KNG&SF) (1957) Werkwijze ter bereiding van een antibioticum met behulp van een Streptomyces-soort. NL Octrooi no 87323 (submitted March 13, 1956; published August 15, 1957)

Kuhn J.O. (2009). Acute oral toxicity study (UDP) in rats, unpublished DSM report, commissioned at Stillmeadow Inc. Study number 12752-09

Kuhn J.O. (2008a). Acute dermal toxicity study in rats, unpublished DSM report, Stillmeadow Inc. Study number 11404-07

Kuhn J.O. (2008b). Acute eye irritation study in rabbits, unpublished DSM report, commissioned at Stillmeadow Inc. Study number 11406-07

Kuhn J.O. (2008c). Acute dermal irritation study in rabbits, unpublished DSM report, commissioned at Stillmeadow Inc. Study number 11407-07

Kuhn J.O. (2008d), Skin sensitization: local lymph node assay in mice, unpublished DSM report, commissioned at Stillmeadow Inc. Study number 11408-07

Ledenbach L.H., Marshall R.T. (2009) Microbiological spoilage of dairy products in *Compendium of the microbiological spoilage of foods and beverages*, Food microbiology and Food safety, W.H. Sperber, M.P. Doyle (eds), Springer Science+Business Media

Levinskas G.J., Ribelin W.E. & Shaffer C.B. (1966), Acute and chronic toxicity of pimaricin, *Toxicol. Appl. Pharmacol.* 8:97-109

Lourens-Hattingh A., Viljoen B.C. (2002), Survival of dairy-associated yeasts in yoghurt and yoghurt-related products, *Food Microbiology*, 19, 597-604

Malten K.E. (1967), Report of an investigation concerning possible allergic side effects of pimaricin in humans. Unpublished DSM report, commissioned at the Instituut voor Geneeskunde en Maatschappij, Nijmegen. Submitted to WHO/JECFA by DSM

Malten K.E. (1968), Report on investigation into possible sensitising side effects of pimaricin in human beings. Unpublished DSM report, commissioned at the Instituut voor Geneeskunde en Maatschappij, Nijmegen. Submitted to WHO/JECFA by DSM

Meerts I.A.T.M. (2002), Evaluation of the ability of natamycin to induce chromosome aberrations in cultured peripheral human lymphocytes, unpublished DSM report, commissioned at Notox project 339356

Montagna M.T., Erroi R., Sanapo S., Caggiano G., Bagordo F., De Donno A. (1998) Food products and fungal contamination. Note I. Preliminary investigation in commercial yoghurt, *Journal of Preventive Medicine and Hygiene*, 39, 68-70

Newcomer V.D., Sternberg T.H., Wright E.T., Reisner R.M., McNall E.G. & Sorensin L.J. (1960), The treatment of systemic diseases with orally administered pimaricin: Preliminary report. *Ann. N.Y. Acad Sci.*, 89: 240-246

Northolt M.D., Van Egmond H.P., Soentoro P. and Deijll E. (1980), Fungal growth and the presence of sterigmatocystin in hard cheese, *Journal- Association of Official Analytical Chemists* 63: 115-119

Raab W.P. (1973), *Natamycin (Pimaricin). Its Properties and Possibilities in Medicine*, Stuttgart: Georg Thieme Publishers

Ray L.L. , Bullerman L.B.(1982), Preventing growth of potentially toxic molds using antifungal agents. *J. Food Protection* 45(10):953-963

- Samson R. A., Stark J. (2007) Expert report on the safety of natamycin and the aspect of resistance, Unpublished DSM report in collaboration with CBS-KNAW fungal biodiversity centre, An institute of the Royal Netherlands Academy of Arts and Sciences
- Scott P.M. (1989), Mycotoxins in dairy products, Ed. H.P van Egmond, Elsevier Applied Science: 193-259. Mycotoxigenic fungal contaminants of cheese and other dairy products
- Shahani K.M., Bullerman L.B., Barahart H.M. and Hortung T.E. (1973), Effect of an antifungal, pimaricin, upon the retardation of food spoilage and incidence of toxins. Proc. 1st International Congress for Bacteriology, Jerusalem, Israel; p. 41
- Shahani K.M., Bullerman L.B., Evans T.A. and Arnold R.G. (1977), Prevention of toxic mold growth in cheese by pimaricin, *Extrait des "Archives de l'Institut Pasteur de Tunis"* 54(3-4):511-520, sponsored by DSM
- Shirling E.B., Gottlieb D. (1972), Cooperative description of type strains of *Streptomyces* V additional descriptions, *Int. J. Syst. Bacteriol.*, 22, 265-394
- Stark J. (2003a), In *Natural antimicrobials for the minimal processing of foods*. Ed. Roller, S, Woodhead Publishing Limited: 82-97, Natamycin, an effective fungicide for food and beverages
- Stark J., Tan H.S. (2003b), In *Food Preservatives, Second Edition*, Ed. Russel N. J., Gould, G. W., Kluwer Academic / Plenum Publishers: 179-195. Natamycin
- Stark J. (2007), In *Food Mycology: a multifaceted approach to fungi and food*. Ed. J. Dijksterhuis and R. A. Samson, CRC Press; Taylor & Francis Group, LLC: 319-331. Cheese and fermented sausages
- Struyk A.P., Hoette I., Drost G., Waisvisz J.M., van Eek T. & Hoogerheide J.C. (1958), Pimaricin, a new antifungal antibiotic. In: Welch, H. & Marti-Ibanez, F., eds, *Antibiotics Annual 1957-1958*, New York: Medical Encyclopedia, Inc., pp. 878-885
- Struyk A.P., Waisvisz J.M. (1975), Pimaricin and process of producing same, US Patent No 3,892,850
- Te Welscher Y.M., Van Leeuwen M.R., De Kruijff B., Dijksterhuis J., Breukink E. (2012), Polyene antibiotic that inhibits membrane transport proteins, *PNAS* Vol 109 (no 28), 11156 - 11159.
- Van Eeken C.J. & Wubs W. (1976), Acute intraperitoneal toxicity of natamycin and three potential metabolites, unpublished internal DSM report No. 15.465, 11 January 1976, submitted to WHO/JECFA by DSM

Van Eeken C.J. van, Birtwhistle R.D.R., Aboulwafa M.J.E. and Hall D.W.R. (1984), Three months study in dogs of the toxicity of natamycin by addition to the food, unpublished internal DSM report no 12.401, submitted to WHO/JECFA by DSM

Van Walbeek W., Scott P.M. and Thatcher F.S. (1968), Mycotoxins from food-borne fungi, *Can. Journal Microbiology* 14 (2):131-137

Var I., Sahan N., Kabak B., Golge O. (2004), The effects of natamycin on the shelf life of yoghurt, *Archiv für Lebensmittelhygiene* 55:1-24

Verspeek-Rip C.M. (2002), Evaluation of the mutagenic activity of natamycin in the *Salmonella typhimurium* reverse mutation assay and the *Escherichia coli* reverse mutation assay (with independent repeat), unpublished DSM report, commissioned at Notox project 339345

Viljoen B.C., Lourens-Hattingh A., Ikalafeng B., Peter G. (2003), Temperature abuse initiating yeast growth in yoghurt, *Food Research International*, 36, 193-197

Williams S.T., Goodfellow M., Alderson G., Wellington E.M.H., Sneath P.H.A., Sackin M.J. (1983), Numerical classification of *Streptomyces* and related genera, *J. Gen. Microbiol.*, 129, 1943-1813

SUBMISSION END