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Original Submission

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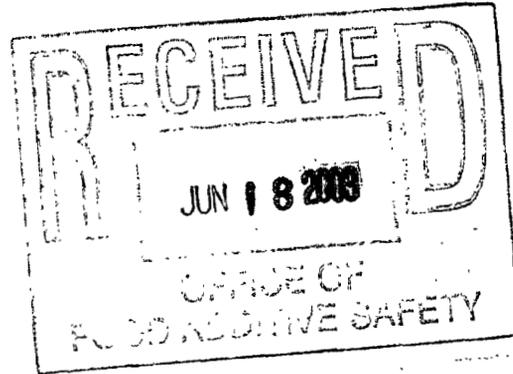
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June 10, 2003

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



**RE: GRAS Notification – Exemption Claim for Lactase Enzyme
Preparation Produced by *Aspergillus niger* that is the Subject
of a GRAS Affirmation Petition, GRASP 3G0016**

Dear Sir or Madam:

Pursuant to proposed 21C.F.R. §§ 170.36(c)(1), 170.36(g)(2), and the Food and Drug Administration ("FDA") preamble discussion concerning the submission of a Generally Recognized As Safe ("GRAS") notification based on a previously filed GRAS affirmation petition, 62 Fed. Reg. 18938, 18953-18954 (April 17, 1997), the Enzyme Technical Association is hereby providing FDA with notice that it has determined, based on scientific procedure, that a lactase enzyme preparation from *Aspergillus niger* (*A. niger*) as a direct human food ingredient is GRAS and therefore exempt from statutory premarket approval requirements. The enzyme from the *A. niger* source organism is also the subject of a GRAS Affirmation Petition 3G0016, which was based on history of use and submitted by the Ad Hoc Enzyme Technical Committee (now known as the Enzyme Technical Association ("ETA")) to the FDA in 1973.

The following information is provided in accordance with the proposed regulation.

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Proposed § 170.36(g)(2)(i): Name and address of the notifier.

Enzyme Technical Association
c/o Gary L. Yingling
1800 Massachusetts Avenue, N.W.
Second Floor
Washington, DC 20036

Proposed § 170.36(g)(2)(ii): The applicable GRAS affirmation petition number.

A GRAS Affirmation Petition for animal-derived, plant-derived, and microbially-derived enzyme preparations was originally submitted by the Ad Hoc Enzyme Technical Committee (now known as ETA) and assigned a petition number, GRASP 3G0016. The FDA filed GRASP 3G0016 on April 12, 1973 (38 Fed. Reg. 9256). The petition was amended on June 12, 1973 (38 Fed. Reg. 15471), August 29, 1984 (49 Fed. Reg. 34305), and June 23, 1987 (52 Fed. Reg. 23607) to include other plant-derived and microbially-derived enzyme preparations. This notification addresses only the lactase enzyme preparation from *A. niger* named above for which FDA action is pending.

Proposed § 170.36(g)(2)(iii): The common or usual name of the substance (i.e., the notified substance).

Lactase from *Aspergillus niger*.

Proposed § 170.36(g)(2)(iv): Applicable conditions of use.

As discussed in greater detail in GRASP 3G0016 as amended, the lactase enzyme preparation is a direct human food ingredient. The use of the enzyme preparation is to catalyze the hydrolysis of the β 1-4 linkage of β -D-galactosides to produce D-galactose and the free sugar to which it was bonded. The *A. niger* derived lactase may be formulated as a liquid or a dry product and is intended for use in the dairy industry to hydrolyze lactose found in milk, whey, cheese, yogurt, and other dairy products.

The enzyme preparation is GRAS for use in food at levels not to exceed Good Manufacturing Practices ("GMPs").

The data and information to support the above uses are contained in GRASP 3G0016, as amended, and the information filed in the attached notification.

Proposed § 170.36(g)(2)(v): Basis for GRAS determination.

The basis for this GRAS determination is through experience based on scientific procedure.

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Proposed § 170.36(g)(2)(vi): Availability of information.

The complete record that supports the GRAS determination has been submitted to the agency in the above referenced GRASP 3G0016, as amended, and the attached notification. Therefore, the complete file is at FDA.

Sincerely,

Alice Cadow, Chair
Enzyme Technical Association

Attachment(s)

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A Lactase Enzyme Preparation Produced by *Aspergillus niger*

A. Introduction

The subject of this notification is a lactase enzyme preparation derived from a pure culture fermentation of a nonpathogenic, nontoxigenic strain of *Aspergillus niger*. This lactase may be formulated as a liquid or dry product and is intended for use in the dairy industry to hydrolyze β -galactosides found in milk, whey, cheese, yogurt and other dairy products. Lactase enzyme preparations are used to reduce the lactose content of milk and milk-derived food products.

β -D-Galactoside galactohydrolase (lactase, EC 3.2.1.23) catalyzes the hydrolysis of terminal, non-reducing β -D-galactose residues in β -D-galactosides. The reaction products of *A. niger* derived lactase action on lactose are glucose and galactose.

The information in the following sections is the basis for our determination of general recognition of safety of a lactase enzyme preparation produced by *A. niger*. Our safety evaluation includes an evaluation of the production strain, the enzyme, and the manufacturing process as well as an evaluation of dietary exposure to the preparation.

The safety of the production organism must be the prime consideration in assessing the probable degree of safety of an enzyme preparation intended for use in food (Pariza and Foster, 1983; Pariza and Johnson, 2001). *A. niger* meets the criteria for nontoxigenicity and nonpathogenicity. *A. niger* has a long history of safe industrial use, is widely distributed in nature, and is commonly used for production of food grade enzymes (Shuster *et al.*, 2002). When the lactase enzyme preparation is manufactured, neither the source organism nor the manufacturing process will introduce impurities into the preparation that may render it unsafe. In addition, the dietary exposure to the lactase preparation does not present a basis for concern about its use.

B. Identity

1. Source
The lactase enzyme preparation is derived from a pure culture of a nonpathogenic, nontoxigenic strain of *Aspergillus niger*.
2. Chemical Name- β -D-Galactoside galactohydrolase
IUB Classification- 3.2.1.23
3. Common or usual name- Lactase
4. CAS Registry Number – 9031-11-2

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5. **Properties**
Lactose, a milk sugar, is a disaccharide comprised of glucose and galactose linked by a β 1-4 glycosidic bond. β -D-Galactoside galactohydrolase catalyses the hydrolysis of this ether bond. The reaction products of *A. niger*-derived lactase action on lactose are glucose and galactose.
6. **Composition**
Like most fungal-derived enzyme preparations used in food processing, the lactase enzyme preparation derived from *A. niger* is not chemically pure, but contains, in addition to the enzyme component, other compounds that derive from the production organism and the fermentation media, residual amounts of processing aids, and substances used as stabilizers, preservatives, or diluents. The *A. niger*-derived lactase is produced using fermentation ingredients and processing aids that are substances acceptable for general use in foods. The lactase enzyme preparation may be formulated as a liquid or a dry product.

C. Manufacturing Process

The lactase enzyme preparation from *A. niger* is manufactured according to procedures outlined by Pariza and Foster (1983) using standard microbial enzyme production technology (Aunstrup, 1979; Aunstrup *et al.*, 1979; Enzyme Applications, 1994) and according to current good manufacturing practice (cGMP).

1. **Raw materials**
The fermentation ingredients used in the manufacture of lactase enzyme preparations are substances that are acceptable for general use in food.
2. **Fermentation process**
The large-scale growth of *A. niger* for the production of lactase can be performed using a liquid medium in a submerged fermentation or the microorganism may be grown on a solid or semi-solid medium held in large trays or drums. With either production method, environmental factors such as pH, temperature, and aeration are controlled. During growth, fermentors are routinely sampled and tested for possible contamination. Should evidence of a significant contamination exist, the batch is rejected.
3. **Recovery process**
The recovery process is a multi-step operation that begins immediately following the fermentation step and involves both the purification and concentration of the product.

4. **Formulation**
Stabilizers, diluents, and/or preservatives that are suitable for general use in food may be added to the lactase enzyme preparation from *A. niger*. The lactase enzyme preparation may be formulated as a liquid or a dry product. The product is standardized according to the product specifications.

D. Specifications

The *A. niger*-derived lactase preparation meets the general and additional requirements for enzyme preparations in the monograph on Enzyme Preparations in the *Food Chemicals Codex* (FCC) (National Research Council, 1996). Lactase assay can be performed using the FCC (National Research Council, 1996) method or by any appropriate validated method.

E. Application

1. **Mode of action**
The lactase derived from *A. niger* catalyzes the hydrolysis of the β 1-4 linkage of β -D-galactosides to produce D-galactose and the free sugar to which it was bonded.
2. **Foods in which used**
The *A. niger* derived lactase may be formulated as a liquid or a dry product and is intended for use in the dairy industry to hydrolyze lactose found in milk, whey, cheese, yogurt and other dairy products.
3. **Level of use**
The lactase enzyme preparation is used in food at minimum levels necessary to achieve the desired effect and according to cGMP.
4. **Enzyme residues in final food**
Lactase enzyme preparations are generally used as processing aids. Residues in food of the lactase derived from *A. niger* will be similar to that of lactase derived from *Kluyveromyces lactis* (21 CFR § 184.1388) (SugarLo, 1984). The lactase enzyme preparation from *A. niger* would not add to the total consumption of lactase from other sources because the enzyme preparation of this notification will be substituted for other lactase enzyme preparations currently in use.

F. Safety Evaluation

1. **Production Organism**
The safety of the production organism must be the prime consideration in assessing the probable degree of safety of an enzyme preparation intended for use in food (Pariza and Foster, 1983; Pariza and Johnson, 2001). If the organism is nontoxic and nonpathogenic, then it is assumed that food

or food ingredients produced from the organism, using current Good Manufacturing Practices, are safe to consume (IFBC, 1990). Pariza and Foster (1983) define a nontoxigenic organism as "one which does not produce injurious substances at levels that are detectable or demonstrably harmful under ordinary conditions of use or exposure" and a nonpathogenic organism as "one that is very unlikely to produce disease under ordinary circumstances". *A. niger* meets these criteria for nontoxigenicity and nonpathogenicity. In addition, *A. niger* is not considered pathogenic by JECFA (1987).

Shuster *et al.*, (2002) reviewed the safety of *A. niger* and describe it as having a very long history of safe industrial use, being widely distributed in nature, and being commonly used for production of food grade enzymes. *A. niger* is generally regarded as a nonpathogenic species Shuster *et al.*, (2002). *A. niger* has been used since 1919 to produce citric acid (Bennett, 2001, Shuster *et al.*, 2002; IFBC, 1990), a commodity chemical, widely used in both the food and pharmaceutical industries. The Food and Drug Administration (FDA) lists *A. niger* as a safe source of citric acid (21 CFR §173.280). Gluconic acid and fumaric acids, though of less economic importance, have also been produced using *A. niger*.

Since at least the 1950s, *A. niger* has become the source of a number of commercially important food enzymes, including glucoamylase, pectinase, lipase hemicellulase, protease, glucose oxidase, and catalase (Beckhorn *et al.*, 1965; Frost and Moss, 1987; Pariza and Johnson, 2002, Bennett 2001, ETA 2002a and b). Carbohydrase and cellulase derived from *A. niger* is approved by the FDA for use in clam and shrimp processing (21 CFR §173.120). Further, FDA lists *A. niger* as a source of enzymes for which it recognized as GRAS in opinion letters issued in the early 1960s (www.cfsan.fda.gov/~dms/opa-enzy.html).

Several studies have been performed where the pathogenic potential of *A. niger* was evaluated. The results of these studies demonstrated that neither ingestion of large doses of spores (Nyireddy *et al.*, 1975) nor inhalation of spores (Bhatia and Mohapatra, 1969) induced mycosis in animals under the conditions of the test.

2. Enzyme

Enzyme proteins themselves do not generally raise safety concerns (FDA, 1992 and 1998; Pariza and Foster, 1983). As indicated in section B, the enzyme preparation of this notification is a β -D-galactoside galactohydrolase, IUB 3.2.1.23, which hydrolyzes the β 1-4 linkages of β -D-galactosides. Lactases have been a component of foods for a long time and thus have been ingested by man for many years (Pfizer, 1996). Several lactase enzyme preparations are considered to be GRAS and have been used in food for at least 20 years. These include the lactase enzyme

preparations from *Candida pseudotropicalis* (21 CFR §184.1387) (Pfizer, 1996), *Kluyveromyces lactis* (previously called *Saccharomyces lactis*) (21 CFR §184.1388) (SugarLo, 1984), and *Kluyveromyces marxianus* (ETA 2002a).

3. **Manufacturing Process**

The lactase enzyme preparation meets the general and additional requirements for enzyme preparations as outlined in the monograph on Enzyme Preparations in the *Food Chemicals Codex*. As described in Section C, the lactase preparation is produced in accordance with current good manufacturing practices, using ingredients that are acceptable for general use in foods, and under conditions that ensure a controlled fermentation. These methods are based on generally available and accepted methods used for production of microbial enzymes (Aunstrup, 1979; Aunstrup *et al.*, 1979, *Enzyme Applications*, 1994).

G. Analytical Methodologies

It is recommended that the *A. niger* lactase enzyme preparation be assayed using the FCC method for lactase (National Research Council, 1996). This assay is based on the measurement of *o*-nitrophenol released over a 10 minutes hydrolysis of *o*-nitrophenyl- β -D-galactopyranoside (ONPG) substrate by lactase at 30 °C and at pH 6.5.

H. Exposure

The estimated dietary exposure to *A. niger*-derived lactase preparation for the proposed use as an enzymatic catalyst in the hydrolysis of lactose and other β -D-galactosides will be similar to that of lactase derived from *Kluyveromyces lactis* (21 CFR § 184.1388) (SugarLo, 1984).

In dairy applications, such as the hydrolysis of lactose, there may be minimal exposure to inactivated enzyme protein. Like other enzymes used as processing aids, the enzyme of this submission will be used at very low levels. Thus in all cases, we conclude that the dietary exposure to the *A. niger* lactase enzyme preparation is negligible and therefore does not present a basis for concern with its use.

I. Conclusions

The evidence provided in this document shows that the lactase enzyme preparation derived from *A. niger* will achieve its intended technical effect. Further, the basic conclusions and guidelines for determining the safety of enzymes used in food and food processing as stated by Pariza and Foster (1983), reiterated by FDA (1998), and updated by Pariza and Johnson (2001) have been applied to this lactase enzyme preparation. When the lactase enzyme preparation is manufactured, neither the source organism (a nonpathogenic, nontoxigenic strain of *A. niger*) nor the manufacturing process will introduce impurities into the

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preparation that may render it unsafe. In addition, the dietary exposure to the lactase preparation does not present a basis for concern about its use.

We conclude, based upon the evaluation of published and unpublished data and information, and based upon scientific procedures (21CFR§170.30(b)), that the lactase enzyme preparation derived from *A. niger* is GRAS for its use as an enzymatic catalyst in the hydrolysis of lactose.

J. References

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End Submission

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