Federal Register, 800 North Capitol Street, NW., suite 700, Washington, DC.

**Effective Date of This AD**

(h) This amendment becomes effective on May 31, 2001.

Issued in Burlington, Massachusetts, on May 7, 2001.

**Francis A. Favara,**

*Acting Manager, Engine and Propeller Directorate, Aircraft Certification Service.*

[FR Doc. 01–12006 Filed 5–15–01; 8:45 am]

**BILLING CODE 4910–13–U**

## DEPARTMENT OF HEALTH AND HUMAN SERVICES

## Food and Drug Administration

## 21 CFR Part 173

**[Docket No. 92F–0396]**

## Secondary Direct Food Additives Permitted in Food for Human Consumption; Alpha-Acetolactate Decarboxylase Enzyme Preparation

**AGENCY:** Food and Drug Administration, HHS.

**ACTION:** Final rule.

**SUMMARY:** The Food and Drug Administration (FDA) is amending the food additive regulations to provide for the safe use of alpha-acetolactate decarboxylase (α-ALDC) enzyme preparation derived from *Bacillus subtilis*, modified by recombinant deoxyribonucleic acid (DNA) techniques to contain the gene coding for α-ALDC from *B. brevis*, for use as a processing aid to produce alcoholic malt beverages and distilled liquors. This action is in response to a petition filed by Novozymes North America, Inc. (formerly Novo Nordisk Bioindustrials, Inc.).

**DATES:** This rule is effective May 16, 2001. Submit written objections and requests for a hearing by June 15, 2001. The Director of the Office of the Federal Register approves the incorporation by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51 of a certain publication in § 173.115(b)(3), effective as of May 16, 2001.

**ADDRESSES:** Submit written objections to the Dockets Management Branch (HFA–305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852.

**FOR FURTHER INFORMATION CONTACT:** Rudaina H. Alrefai, Center for Food Safety and Applied Nutrition (HFS–206), Food and Drug Administration, 200 C St. SW., Washington, DC 20204, 202–418–3034.

**SUPPLEMENTARY INFORMATION:** In a notice published in the **Federal Register** of November 30, 1992 (57 FR 56585), FDA announced that a food additive petition (FAP 2A4345) had been filed by Novo Nordisk Bioindustrials, Inc., later renamed Novozymes North America, Inc., 77 Perry Chapel Church Rd., P.O. Box 576, Franklinton, NC 27525. The petition proposed that the food additive regulations be amended to provide for the safe use of α-acetolactate decarboxylase (ALDC) derived from *B. subtilis* modified by recombinant DNA techniques to contain the gene coding for ALDC from *B. brevis* for use as a processing aid in the brewing and alcohol industries.

When FDA filed the petition in the **Federal Register** of November 30, 1992 (57 FR 56585), it contained an environmental assessment (EA). The notice of filing stated '' * * * if the agency finds that an environmental impact statement is not required and this petition results in a regulation, the notice of availability of the agency's finding of no significant impact and the evidence supporting that finding will be published with the regulation * * *.'' In the **Federal Register** of July 29, 1997 (62 FR 40570), FDA published a final rule on its National Environmental Policy Act policies and procedures, which became effective on August 28, 1997. In a letter dated January 4, 2001, the petitioner submitted a claim of categorical exclusion under 21 CFR 25.32(k). The agency has reviewed the claim of categorical exclusion and has concluded that it is warranted.

### I. Evaluation of Safety of the Petitioned Use of the Additive

*A. Introduction*

The use of α-ALDC enzyme preparation from *B. subtilis* is to prevent the formation of diacetyl that causes unpleasant taste in beer and other alcoholic beverages. The enzyme α-ALDC is to be distinguished from the α-ALDC enzyme preparation, which contains α-ALDC as the principal active component in addition to other components derived from the production organism and fermentation media. This document will refer to the former as ''α-ALDC'' and the latter as ''α-ALDC enzyme preparation.'' Diacetyl is normally formed from α-acetolactate during fermentation. Alpha-ALDC, which is the active component of the petitioned enzyme preparation, catalyzes the conversion of α-acetolactate directly to acetoin, thereby reducing the time needed for spontaneous degradation of diacetyl to acetoin.

*B. Host Organism*

The host organism, *B. subtilis*, for production of α-ALDC is widely distributed in nature and is commonly present in foods eaten by both humans and animals. It also has a history of safe use as a source of enzymes in food enzyme manufacturing industry prior to 1958. Thus, *B. subtilis* is considered to be a nonpathogenic microorganism.

*C. Donor Organism*

*B. brevis* is the microorganism used as the source of the genetic material for the α-ALDC enzyme that is the subject of FAP 2A4345. FDA reviewed the safety of the DNA that encodes the enzyme α-ALDC from *B. brevis* and the enzyme it produces (discussed below), because only that DNA is transferred to the host strain from the donor organism.

*D. Production Organism*

The petitioner provided information demonstrating that the plasmid carrying the gene for α-ALDC is stably integrated into the chromosome of *B. subtilis* production strain. The petitioner conducted a study to evaluate the pathogenicity of three *B. subtilis* strains. In this study, mice received an intraperitoneal injection with the *B. subtilis* host strain, *B. subtilis* production strain, and a *B. subtilis* strain capable of producing α-ALDC but not used as a source of the petitioned enzyme preparation. FDA reviewed this study as well as the scientific literature concerning potential pathogenicity of *B. subtilis* and did not identify any microbiological concern (Refs. 1, 2, and 3).

*E. Enzyme Preparation*

The α-ALDC enzyme preparation is manufactured by a submerged pure culture fermentation of a genetically engineered strain of *B. subtilis* carrying the *B. brevis* gene that encodes α-ALDC. The enzyme is secreted to the fermentation broth and processed by removing the cellular debris, followed by concentration and formulation. For certain applications, the α-ALDC enzyme preparation is stabilized by crosslinking with glutaraldehyde (referred to as d-ALDC).

The petitioner submitted several toxicological studies that address the safety of the petitioned α-ALDC and d-ALDC enzyme preparations. These include: A teratogenicity study in rats and genotoxicity studies, including tests for mutagenic activity in *Salmonella typhimurium* and mammalian cells, as well as tests for chromosome-damaging activity in human lymphocytes. FDA has reviewed these studies and concludes that the petitioned α-ALDC

enzyme preparation does not raise any toxicity concerns at the expected level of consumption or have any mutagenic potential (Refs. 4 and 5).

*F. Source of Impurities*

Enzyme preparations used in food are usually not chemically pure, but contain cellular and processing material. The nature and amounts of these impurities in the finished enzyme preparation depend on the organism from which the enzyme preparation is produced (the production organism), the fermentation materials and methods used to grow the production organism, and the materials and methods used to generate the finished enzyme preparation. Thus, the question is whether the production organism or the manufacturing methods used to grow the production organism or to generate the finished enzyme preparation from recombinant *B. subtilis* will introduce impurities that raise concerns about the safety of the enzyme preparation.

One issue raised by the use of recombinant DNA techniques is the potential transfer of DNA encoding for extraneous proteins along with the gene of interest (i.e., α-ALDC), thereby contaminating the enzyme preparation. As a matter of current good manufacturing practice, manufacturers using recombinant DNA technology must ensure that they have not inadvertently cloned extraneous protein-encoding DNA along with the α-ALDC gene that may lead to contamination of the α-ALDC enzyme preparation. Such assurance can come from reviewing the details of the cloning steps, which include the origin and sequence of the DNA fragments used in the cloning, and full characterization of the final genetic constructs via techniques such as DNA sequencing. The petition contains information demonstrating that the petitioner evaluated the cloning process to ensure that the final cloning product, i.e., the DNA with the α-ALDC gene, used in the development of the recombinant *B. subtilis* was accurately constructed. The petitioner submitted evidence to demonstrate that it cloned a full-length copy of the α-ALDC gene from *B. brevis* into *B. subtilis*. The petitioner also described the multistep process for constructing the *B. subtilis* production strain. These steps involve the use of several plasmids (intermediate plasmids) that confer resistance to chloramphenicol and kanamycin, both of which are clinically useful antibiotics. Through various techniques, these plasmids are eliminated during the construction of the gene encoding α-ALDC. The petitioner tested the final enzyme preparation for the presence of the production strain or other microbial activity and for antibacterial activity and reported none present. Therefore, the agency concludes that the production strain is effectively removed by the enzyme purification procedure. Furthermore, the α-ALDC enzyme preparation conforms to the general and additional requirements for enzyme preparations in the *Food Chemicals Codex* (Ref. 6) and does not contain the production organism or antimicrobial activity.

FDA concludes that, when the α-ALDC enzyme preparation is manufactured in conformity with § 173.115, there is no basis for concern regarding the possibility that the α-ALDC enzyme preparation will be contaminated by the products of extraneous genetic material inserted along with the α-ALDC gene in *B. subtilis* (Ref. 1). Furthermore, FDA concludes, having considered the evidence concerning the production organism and the processing steps to derive the α-ALDC enzyme preparation, that: (1) *B. subtilis* containing α-ALDC gene from *B. brevis* is safe for use as a source of food-grade α-ALDC enzyme preparation, (2) impurities resulting from the use of *B. subtilis* containing α-ALDC gene from *B. brevis* in the production of α-ALDC enzyme preparation will not affect the safety of the α-ALDC enzyme preparation, and (3) processing aids and their impurities that are used to make the commercial α-ALDC enzyme preparation and that may remain in food processed with this enzyme preparation present no safety concerns (Refs. 5 and 7).

## II. Conclusion

FDA has evaluated the data in the petition and other relevant material. Based on this information, the agency concludes that: (1) The proposed use of α-ALDC enzyme preparation from *B. subtilis* containing the α-ALDC gene from *B. brevis* is safe, (2) the additive will achieve its intended technical effect, and (3) the regulations in § 173.115 should be amended as set forth below in this document.

## III. Inspection of Documents

In accordance with § 171.1 (21 CFR 171.1(h)), the petition and the documents that FDA considered and relied upon in reaching its decision to approve the petition are available for inspection at the Center for Food Safety and Applied Nutrition by appointment with the information contact person listed above. As provided in § 171.1(h), the agency will delete from the documents any materials that are not available for public disclosure before making the documents available for inspection.

## IV. Environmental Impact

The agency has determined under 21 CFR 25.32(k) that this action is of a type that does not individually or cumulatively have a significant effect on the human environment. Therefore, neither an EA nor an environmental impact statement is required.

## V. Paperwork Reduction Act of 1995

This final rule contains no collection of information. Therefore, clearance by the Office of Management and Budget under the Paperwork Reduction Act of 1995 is not required.

## VI. Objections

Any person who will be adversely affected by this regulation may at any time file with the Dockets Management Branch (address above) written objections by June 15, 2001. Each objection shall be separately numbered, and each numbered objection shall specify with particularity the provisions of the regulation to which objection is made and the grounds for the objection. Each numbered objection on which a hearing is requested shall specifically so state. Failure to request a hearing for any particular objection shall constitute a waiver of the right to a hearing on that objection. Each numbered objection for which a hearing is requested shall include a detailed description and analysis of the specific factual information intended to be presented in support of the objection in the event that a hearing is held. Failure to include such a description and analysis for any particular objection shall constitute a waiver of the right to a hearing on the objection. Three copies of all documents are to be submitted and are to be identified with the docket number found in brackets in the heading of this document. Any objections received in response to the regulation may be seen in the Dockets Management Branch between 9 a.m. and 4 p.m., Monday through Friday.

## VII. References

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

1. Memorandum from W. Koch, FDA, to L. Kahl, FDA, September 23, 1993.

2. Memorandum from J. Madden, FDA, to the Biotechnology Policy Branch, FDA, August 29, 1995.

3. Memorandum from J. Madden, FDA, to the Biotechnology Policy Branch, FDA, October 20, 1995.
4. Memorandum from R. D. Benz, FDA, to K. C. Raffaele, FDA, July 20, 1995.
5. Memorandum from the Division of Health Effects Evaluation, FDA, to R. H. Alrefai, FDA, May 4, 1999.
6. *Food Chemicals Codex*, 1996, 4th ed., National Academy Press, Washington, DC, pp. 133–134.
7. Memorandum from the Division of Health Effects Evaluation, FDA, to R. H. Alrefai, FDA, August 20, 1999.

**List of Subjects in 21 CFR Part 173**

Food additives, Incorporation by reference.

Therefore, under the Federal Food, Drug, and Cosmetic Act and under authority delegated to the Commissioner of Food and Drugs, 21 CFR part 173 is amended as follows:

## PART 173—SECONDARY DIRECT FOOD ADDITIVES PERMITTED IN FOOD FOR HUMAN CONSUMPTION

1. The authority citation for 21 CFR part 173 continues to read as follows:

**Authority:** 21 U.S.C. 321, 342, 348.

2. Section 173.115 is added to subpart B to read as follows:

**§ 173.115 Alpha-acetolactate decarboxylase (α-ALDC) enzyme preparation derived from a recombinant Bacillus subtilis.**

The food additive alpha-acetolactate decarboxylase (α-ALDC) enzyme preparation, may be safely used in accordance with the following conditions:

(a) The food additive is the enzyme preparation derived from a modified *Bacillus subtilis* strain that contains the gene coding for α-ALDC from *Bacillus brevis*.

(b)(1) The manufacturer produces the additive from a pure culture fermentation of a strain of *Bacillus subtilis* that is nonpathogenic and nontoxigenic in man or other animals.

(2) The manufacturer may stabilize the enzyme preparation with glutaraldehyde or with other suitable approved food additives or generally recognized as safe substances.

(3) The enzyme preparation must meet the general and additional requirements for enzyme preparations in the *Food Chemicals Codex*, 4th ed., 1996, pp. 133–134, which is incorporated by reference. The Director of the Office of the Federal Register approves this incorporation by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies may be obtained from the National Academy Press, 2101 Constitution Ave. NW., Washington, DC 20055, or may be examined at the Center for Food Safety and Applied Nutrition, 200 C St. SW., rm. 3321, Washington, DC, or at the Office of the Federal Register, 800 North Capitol St. NW., suite 700, Washington, DC.

(c) The additive is used in an amount not in excess of the minimum required to produce its intended effect as a processing aid in the production of alcoholic malt beverages and distilled liquors.

Dated: May 4, 2001.

**Margaret M. Dotzel,**

*Associate Commissioner for Policy.*

[FR Doc. 01–12225 Filed 5–15–01; 8:45 am]

BILLING CODE 4160–01–F

## DEPARTMENT OF HEALTH AND HUMAN SERVICES

### Food and Drug Administration

### 21 CFR Part 558

### New Animal Drugs For Use in Animal Feeds; Narasin, Nicarbazin, and Bambermycins

**AGENCY:** Food and Drug Administration, HHS.

**ACTION:** Final rule.

**SUMMARY:** The Food and Drug Administration (FDA) is amending the animal drug regulations to reflect approval of a new animal drug application (NADA) filed by Elanco Animal Health. The NADA provides for use of approved narasin/nicarbazin and bambermycins Type A medicated articles to make three-way combination Type C medicated feeds used for prevention of coccidiosis, increased rate of weight gain, and improved feed efficiency in broiler chickens.

**DATES:** This rule is effective May 16, 2001.

**FOR FURTHER INFORMATION CONTACT:** Charles J. Andres, Center for Veterinary Medicine (HFV–128), Food and Drug Administration, 7500 Standish Pl., Rockville, MD 20855, 301–827–1600.

**SUPPLEMENTARY INFORMATION:** Elanco Animal Health, A Division of Eli Lilly & Co., Lilly Corporate Center, Indianapolis, IN 46285, filed NADA 140-942 that provides for use of Maxiban® (36 grams per pound (g/lb) each of narasin and nicarbazin) and Flavomycin® (2, 4, or 10 g/lb of bambermycins activity) Type A medicated articles to make three-way combination Type C medicated feeds for use in broiler chickens. The combination Type C medicated feeds contain 27 to 45 g/ton narasin and 27 to 45 g/ton nicarbazin (in a fixed 1:1 ratio) and 1 to 2 g/ton bambermycins, and are used for prevention of coccidiosis caused by *Eimeria tenella*, *E. necatrix*, *E. acervulina*, *E. brunetti*, and *E. mivati*, and for increased rate of weight gain and improved feed efficiency. The NADA is approved as of March 8, 2001, and the regulations are amended in 21 CFR 558.95 and 558.363 to reflect the approval. The basis of approval is discussed in the freedom of information summary

In accordance with the freedom of information provisions of 21 CFR part 20 and 514.11(e)(2)(ii), a summary of safety and effectiveness data and information submitted to support approval of this application may be seen in the Dockets Management Branch (HFA–305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852, between 9 a.m. and 4 p.m., Monday through Friday.

The agency has determined under 21 CFR 25.33(a)(2) that this action is of a type that does not individually or cumulatively have a significant effect on the human environment. Therefore, neither an environmental assessment nor an environmental impact statement is required.

This rule does not meet the definition of ''rule'' in 5 U.S.C. 804(3)(A) because it is a rule of ''particular applicability.'' Therefore, it is not subject to the congressional review requirements in 5 U.S.C. 801–808.

**List of Subjects in 21 CFR Part 558**

Animal drugs, Animal feeds.

Therefore, under the Federal Food, Drug, and Cosmetic Act and under authority delegated to the Commissioner of Food and Drugs and redelegated to the Center for Veterinary Medicine, 21 CFR part 558 is amended as follows:

## PART 558—NEW ANIMAL DRUGS FOR USE IN ANIMAL FEEDS

1. The authority citation for 21 CFR part 558 continues to read as follows:

**Authority:** 21 U.S.C. 360b, 371.

**§ 558.95 [Amended]**

2. Section 558.95 *Bambermycins* is amended in paragraph (d)(5)(iv) by adding ''nicarbazin or'' following ''with'', and in paragraph (d)(5)(v) by removing ''Nicarbazine'' and adding in its place ''Nicarbazin''.

3. Section 558.363 is amended by adding paragraph (d)(1)(xii) to read as follows:

**§ 558.363 Narasin.**

* * * * *