Rules and Regulations

Federal Register
Vol. 58, No. 87
Friday, May 7, 1993

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

21 CFR Part 184

[Docket No. 89G–0384]

Direct Food Substances Affirmed as Generally Recognized as Safe; Chymosin Enzyme Preparation Derived From Aspergillus Niger Van Tieghem Variety Awamori (Nakazawa) Al-Musallam

AGENCY: Food and Drug Administration, HHS.

ACTION: Final rule.

SUMMARY: The Food and Drug Administration (FDA) is amending its regulations to affirm that the use of chymosin preparation derived by fermentation from genetically modified *Aspergillus niger* van Tieghem variety *awamori* (Nakazawa) Al-Musallam (*A. niger* var. *awamori*) is generally recognized as safe (GRAS). This action is in response to a petition filed by Genencor, Inc., now Genencor International, Inc.

EFFECTIVE DATE: May 7, 1993.

FOR FURTHER INFORMATION CONTACT: Vincent E. Zenger, Center for Food Safety and Applied Nutrition (HFS–206), Food and Drug Administration, 200 C St. SW., Washington, DC 20204, 202–254–9523.

SUPPLEMENTARY INFORMATION:

I. Background

In accordance with the procedures described in § 170.35 (21 CFR 170.35), Genencor International, Inc. (Genencor), previously known as Genencor, Inc., 180 Kimball Way, South San Francisco, CA 94080, submitted a petition (GRASP 9G0352) requesting that its chymosin preparation (referred to as "chymosin" in the notice of filing of the Genencor petition that FDA published in the Federal Register of October 4, 1989 (54 FR 40910)) be affirmed as GRAS as a direct human food ingredient. Genencor's chymosin preparation is derived from the fermentation of genetically modified *A. niger* var. *awamori*. Chymosin is the principal enzyme in rennet, a GRAS food ingredient that is used for its milk-clotting activity and that is primarily responsible for that activity. Chymosin preparation is intended for use as a substitute for rennet.

To avoid confusion between chymosin, the enzyme, and the chymosin-containing enzyme preparation (in which chymosin is the principal active component, but which may also contain impurities), this document will henceforth use the terms "chymosin" to refer to the enzyme and "chymosin preparation" to refer to the fermentation-derived chymosin enzyme preparation.

In the October 4, 1989, notice of filing, FDA gave interested parties an opportunity to submit comments concerning the subject chymosin preparation to the Dockets Management Branch (HFA–305), Food and Drug Administration, rm. 1–23, 12420 Parklawn Dr., Rockville, MD 20857. FDA received three comments in response to the notice of filing. One of the comments offered a general opinion on the regulation of biotechnology-derived food ingredients, and the second expressed support for approval of the petition. Neither of these comments contained information relevant to the safety, functionality, environmental impact, or GRAS status of the food use of the chymosin preparation. The third comment, however, raised two issues that concern the evaluation of the chymosin preparation: (1) Whether the petitioner has incorrectly identified the host organism *A. niger* var. *awamori*; and (2) whether, because 10 percent of Genencor's chymosin is glycosylated, the safety of the glycosylated material itself must be evaluated on its own merit. The agency's evaluation of these issues is discussed later in this document.

II. Standards For GRAS Affirmation

Pursuant to § 170.30 (21 CFR 170.30), general recognition of safety may be based only on the views of experts qualified by scientific training and experience to evaluate the safety of substances. The basis of such views may be either: (1) Scientific procedures; or (2) in the case of a substance used in food prior to January 1, 1958, experience based on common use in food. General recognition of safety based upon scientific procedures requires the same quantity and quality of scientific evidence as is required to obtain approval of a food additive regulation and ordinarily is to be based upon published studies, which may be corroborated by unpublished studies and other data and information (§ 170.30(b)). As shown below, FDA has evaluated Genencor's petition on the basis of scientific procedures to establish that the petitioned chymosin preparation is GRAS.

Rennet is an animal-derived enzyme preparation that is GRAS as specified in § 184.1685 (21 CFR 184.1685). Therefore, if published information shows that the principal active component of the chymosin preparation is the same as that of rennet, and that the other components (i.e., impurities) of the chymosin preparation, which may differ from the other components (i.e., impurities) of rennet, do not render the use of the substance unsafe, then the chymosin preparation derived from *A. niger* var. *awamori* would present no more safety concern than rennet. In such circumstances, FDA can affirm the chymosin preparation derived from *A. niger* var. *awamori* as GRAS for use as a replacement for rennet.

III. Safety

A. Introduction

Chymosin, also known as rennin, is the principal milk-clotting enzyme present in rennet (Ref. 1). Rennet is an enzyme preparation that will clot milk, forming it into curds and whey (Refs. 1 and 2). It is used to make cheese and other dairy products. Rennet has a long and extensive history of safe use in food and has been affirmed by FDA as GRAS in § 184.1685 (48 FR 51151, November 7, 1983).

Food-grade rennet is an enzyme preparation that is isolated from the fourth stomach of calves, kids, or lambs. Commercially, it is generally derived by aqueous extraction from the fourth stomach of unweaned calves. The aqueous extraction step is followed by purification steps and an acidification step to cleave prochymosin (the inactive

precursor of chymosin) in rennet into chymosin (Ref. 1).

There are two predominant forms of calf chymosin, chymosin A and chymosin B (Ref. 1). Foltmann et al. have shown that chymosin A and chymosin B differ by a single amino acid (Ref. 3). In this document, the term "chymosin" refers to either, or both, chymosin A and chymosin B.

Techniques developed in the early 1970's (frequently termed "recombinant DNA technology" or "cloning techniques") enable scientists to locate and to obtain a segment of deoxyribonucleic acid (DNA) containing a gene of interest. Scientists are also able to move that DNA segment into a vector (a DNA molecule that is easy to manipulate) and then introduce that segment into a new host organism where the segment can be correctly expressed (that is, produce the protein that it would have produced in the original organism). These techniques are well-known to molecular biologists (Refs. 4 and 5).

B. The Chymosin Component

Using cloning techniques, scientists in a number of different laboratories have identified the gene in the calf from which the chymosin in rennet is produced, i.e., the prochymosin gene (Refs. 6 through 8). Scientists have moved the calf prochymosin gene into *A. niger* var. *awamori* (Refs. 9 through 12) as well as into other microorganisms (Refs. 6 through 8 and 13 through 17).

These scientists have used a variety of techniques to demonstrate that they have cloned full-length copies of the correct gene. Such techniques include: (1) DNA sequencing, whereby the cloned putative prochymosin gene was shown to have the nucleotide sequence that encodes the amino acid sequence of prochymosin (Refs. 6 through 8); (2) nucleic acid hybridization, whereby the cloned DNA fragments or the ribonucleic acid (RNA) molecules transcribed from the DNA fragments were shown to hybridize (i.e., specifically bind) with complementary DNA in the prochymosin gene (Refs. 7, 8, and 13 through 16); and (3) physical mapping, whereby the cloned DNA segments were shown to be large enough to contain the prochymosin gene and, when digested with appropriate DNA-cutting enzymes and the resulting DNA fragments separated by size, were shown to yield the pattern of DNA fragments expected for the prochymosin gene (Refs. 7, 8, and 13 through 17).

The published evidence establishes that the new host organisms are able to use the prochymosin gene to produce prochymosin that has the same molecular weight as prochymosin found in calf rennet (Refs. 11 through 15 and 17 through 19). This evidence also establishes that the prochymosin that is produced (cloned prochymosin) can be cleaved into chymosin (cloned chymosin) that has the same molecular weight and the same functional activity as chymosin found in calf rennet (Refs. 11, 13, 14, and 16 through 19).

A number of techniques have been used to demonstrate that the chymosin produced by *A. niger* var. *awamori* is equivalent to calf chymosin, including: (1) Reaction with antibodies to calf chymosin, (2) amino acid composition analysis, (3) amino acid sequencing, and (4) specific activity determination (Refs. 9 through 12). The molecular weight of chymosin was determined, using sodium dodecyl sulfate polyacrylamide gel electrophoresis and tricine-sodium dodecyl sulfate polyacrylamide gel electrophoresis, techniques that enable one to determine the comparative molecular weight of proteins, based on their rate of migration through the gels. Cloned chymosin was found to migrate through these gels at the same rate as the chymosin found in rennet (Refs. 9 and 11), except for a minor band representing 10 percent of the molecules that are glycosylated (see discussion below) and that have a slightly higher molecular weight.

The functional activity of chymosin that was measured was milk-clotting activity. Cloned chymosin was found to clot milk at the same rate as the chymosin in rennet under various temperatures, salt concentrations, and pH conditions (Refs. 11 and 13 through 21).

One of the comments FDA received in response to the **Federal Register** notice announcing the filing of the GRAS petition (54 FR 40910) questioned whether the fact that about 10 percent of chymosin produced by *A. niger* var. *awamori* is glycosylated presents any threat to human safety. The comment urged FDA to give careful consideration to the glycosylation issue. However, the comment did not present any information on the nature of any adverse effect that might result from the ingestion of a small amount of this glycosylated enzyme. In fact, a variety of proteins, including many glycosylated protein enzymes, are present in foods that have been safely consumed for many years (Ref. 22 through 24). For example, chymosin derived from lamb, which has been affirmed as GRAS (§ 184.1685), is a glycosylated enzyme (Ref. 25), as are other milk-clotting enzymes listed in FDA regulations (§ 173.150). Therefore, the agency finds that glycosylation of a small proportion of an enzyme does not per se raise any safety concerns. Such a conclusion is consistent with current scientific thinking that enzymes that are substantially similar to proteins known to be safely consumed (including minor variations in structure such as glycosylation) do not raise new safety concerns (see, for example, Ref. 26).

Based on the fact that published information demonstrates that chymosin produced from the cloned chymosin gene has the same molecular weight and the same functional activity as the chymosin derived from calves, FDA concludes that the chymosin enzyme in the chymosin preparation is equivalent to the chymosin enzyme in calf rennet. Therefore, FDA concludes that the chymosin enzyme in the chymosin preparation is as safe as the chymosin enzyme in rennet.

Moreover, corroborative evidence of the safety of the petitioned chymosin enzyme preparation, including glycosylated chymosin, is provided by the results of a feeding study conducted by the petitioner. In that study, described later in this document, no adverse effects attributable to consumption of the enzyme preparation were noted.

C. Sources of Impurities

Enzyme preparations used in food processing are usually not chemically pure, but contain extraneous source (cellular) and processing material. The nature and amounts of these materials in the finished enzyme preparation depend on the organism from which the enzyme is produced (i.e., the source or 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.

Both the source material and the manufacturing methods for producing the chymosin preparation differ from those used to produce animal rennet. Therefore, the impurities in the chymosin preparation will differ from those in rennet. The question thus is whether the source material or manufacturing methods for the chymosin preparation will introduce impurities that would raise concerns about the safety of the preparation.

1. Processing steps

Researchers in several laboratories have published papers containing descriptions of methods that they used for producing chymosin preparations from microorganisms containing the calf prochymosin gene (Refs. 13 through 19 and 27 through 30). The enzyme that is the subject of this petition is secreted

from the production organism during fermentation and, therefore, is an extracellular enzyme product. Thus, it is not necessary to disrupt the cells to recover the enzyme. Extracellular enzymes account for approximately three-fourths of the market for fermentation-derived enzymes, and the techniques for their production and processing are well-known (Refs. 31 and 32). The processing methods developed by Genencor and described in this petition and in the published literature (Refs. 29 and 30) do not differ in any significant way from the published methods generally used to produce extracellular enzymes. The key steps described by Genencor are summarized below.

A. niger var. *awamori* is grown in liquid nutrient medium until the desired activity of chymosin is reached. The broth is then treated with acid to interrupt the fermentation and kill the cells of the production organism. The acid treatment step also degrades any DNA which may be present in the broth. The broth is filtered, which removes the cell material from the chymosin-containing filtrate. Chymosin is then recovered from the filtrate, using one of two methods. In one method, the filtrate is passed through a chromatography column. Chymosin binds to the chromatographic resin while impurities pass through the column. Chymosin is then eluted from the column with an appropriate solution. In another method, chymosin is extracted from the filtrate and purified by ion exchange chromatography. Chymosin recovered by either method is formulated to the desired strength and sterilized by filtration.

FDA finds that the manufacturing method described does not require the use of any processing materials that are not GRAS or are not approved food additives. Accordingly, the agency is specifying in the amended regulation that the substance being affirmed as GRAS is one that is produced using only processing materials that are GRAS substances or food additives approved for use in this type of process.

Therefore, the agency concludes that the manufacturing steps will not introduce impurities into the enzyme preparation that will adversely affect the safety of the preparation.

2. Production organism

The petitioner presented information that the source for the chymosin enzyme preparation that is the subject of this petition is the production organism "*Aspergillus niger* var. *awamori*." The full proper name of this organism is *Aspergillus niger* van Tieghem variety *awamori* (Nakazawa) Al-Musallam. To identify clearly the source organism that has been evaluated in this review, the agency will use the full proper name in the regulation set forth below. However, for simplicity, the organism is referred to as *A. niger* var. *awamori* in the preamble of this document. The agency notes that the organism is also referred to as *Aspergillus awamori* Nakazawa (*Aspergillus awamori*).

One of the comments received in response to the filing notice stated that the name *A. niger* var. *awamori* incorrectly identifies the production organism. The comment contended that characterizing the production organism as a variety of *A. niger* implies that the production organism has a history of safe use in producing enzymes for use in food, when in fact it should be characterized as a distinct species, *A. awamori*, which species has no such history.

FDA notes that it is relying on scientific procedures, and not on history of safe use, as a basis for affirming the general recognition of safety of Genencor's chymosin preparation. In reviewing the petition, FDA evaluated whether the production organism is adequately identified and whether the scientific information that supports safety pertains to the production organism. In its evaluation, FDA relied on information that refers to the production organism and did not rely on information regarding other members of the genus *Aspergillus*.

The taxonomic placement and name of an organism may change as a result of scientific advances. This is especially true for organisms such as *A. niger* var. *awamori* whose taxonomic placement is based on highly variable characteristics such as colony color (Refs. 33 and 34). If internationally accepted rules of nomenclature are followed, changes in the taxonomic placement of an organism should not affect the ability to identify scientific references to the organism of interest, including scientific references to its toxigenicity, pathogenicity, or use in the production of food or enzymes.

The production organism for this enzyme preparation is a fungus. FDA notes that the proper naming of fungi should follow the internationally accepted rules of nomenclature used for plants (Ref. 36). Proper scientific reference to a fungal species is done with its Latin binomial, representing the genus in which the species has been placed and the specific epithet; the name the Latin binomial of the author who introduced the specific epithet should follow. If a species has been moved in its taxonomic placement, the Latin binomial may be followed by the name of the author who made the original type description in parenthesis, followed by the name of the author of the current combination of generic and specific epithet. Proper use of nomenclature allows references to a particular organism to be followed historically in the scientific literature.

The organism referred to by the petitioner as *A. niger* var. *awamori* was first described in 1907 and was named *Aspergillus awamori* Nakazawa. Since the original description, authoritative texts have continued to refer to *A. awamori* as a distinct species (Refs. 36 through 38). However, this organism, along with many others in the genus *Aspergillus*, has been reclassified. A monograph on the taxonomy of the black *Aspergilli* was published in 1980. In that monograph, based on an analysis of 28 taxonomic characteristics, Al-Musallam (Ref. 33) reclassified the production organism as *Aspergillus niger* van Tieghem variety *awamori* (Nakazawa) Al-Musallam. More recent molecular analytical approaches to taxonomy, including DNA restriction fragment length polymorphism analysis, have confirmed this taxonomic placement (Refs. 34 and 39). Genencor also submitted evidence that this taxonomic placement is recognized by experts in the field (Ref. 40).

In evaluating published information, FDA recognized that prior to 1980, the production organism was referred to as *A. awamori* and occasionally as *A. niger* type *awamori*, and that literature published prior to 1980 uses these names when referring to the production organism. Literature published after 1980 may refer to the production organism as *A. niger* var. *awamori* or one of its synonyms, for example, *A. awamori* Nakazawa. Based on an analysis of the data submitted, FDA finds sufficient evidence to support the identification of the petitioner's production organism as *Aspergillus niger* van Tieghem variety *awamori* (Nakazawa) Al-Musallam (synonym *Aspergillus awamori* Nakazawa), and further concludes that the references relied on by the agency pertain to the production organism.

The petitioner provided published information documenting that the production organism has been safely used in a variety of food and food enzyme applications. These data provide a context for FDA's safety evaluation, and are useful in determining what quantity of data are necessary to establish the safety of the organism. *A. niger* var. *awamori* (referred to in these references as *A. awamori*) has been identified and isolated from the starter cultures used to

produce several types of fermented rice alcoholic products (Refs. 38 and 41 through 47) and has also been used in making other fermented food products. Reed (Ref. 48) reports its use in sorghum fermentations and in brewing. It is cited in patents for making Worcester sauce (Ref. 43) and making "mirin," which can be used as a seasoning or dark liquor (Ref. 44). It has also been referenced as the production organism for citric acid produced by fermentation (Refs. 49 and 50).

There are numerous references in the literature to the use of *A. niger* var. *awamori* as the source organism for production of a variety of enzymes, including glucoamylase (Ref. 51) and α-amylase (Ref. 48). For example, Raper and Fennell (Ref. 36) cite two uses of *A. niger* var. *awamori* for enzyme production; one of these was reported by Komaki in 1956 to 1957 as a source of amylase used for the production of glucose from starch, under the synonym *A. usamii*, and the other was reported by Feniksova and Shilova in 1960 as a source of enzymes for production of glucose syrup from starch. Hiram Walker's patent for production of distiller's yeast (Ref. 52) cites the use of glucoamylase from *A. niger* var. *awamori* in treating grain mash. Hiram Walker deposited this strain with the Agricultural Research Station Culture Collection, Northern Regional Research Laboratory (NRRL) where it was listed as *Aspergillus awamori* strain NRRL 3112. This strain is the source organism for a commercial glucoamylase sold since 1963 (Ref. 53). This same strain, NRRL 3112, is also the strain Genencor genetically manipulated to create the final production organism.

The petitioner provided several published animal studies supporting the organism's safety. Semeniuk et al., (Ref. 54) studied the toxigenicity of 392 strains of *Aspergillus* obtained from the Agricultural Research Station Culture Collection of NRRL. The strains studied included three different strains of *A. niger* var. *awamori*. The investigators fed each strain to chicks and mice for 4 weeks. While some of the 392 *Aspergillus* strains they tested were found to be toxic, all three of the *A. niger* var. *awamori* strains were found to be nontoxic. In addition, Bogoroditskaya and Dyubyuk (Ref. 55) reported that amyloprotease prepared from *A. niger* var. *awamori* was nontoxic when administered orally in acute doses of up to 5 grams per kilogram to mice and guinea pigs.

Species of the *A. niger* group are not considered to be of primary significance in the cause of disease (Refs. 56 through 59). During the period 1946 to 1965, however, reports appeared in which organisms identified as *A. niger* var. *awamori* were isolated from several infected postoperative wounds and ear lesions (Ref. 56). There is general agreement that reduced host resistance is required in order for the fungal infection to become established (Refs. 56 through 58), and in many cases there is doubt as to whether *A. niger* var. *awamori* was the causative agent of the infection (Ref. 58). None of the cases of infection was the result of ingestion of *A. niger* var. *awamori*. Moreover, the processing steps described above, including acid treatment and filtration, remove any viable cells of the production organism from the final product, which eliminates the possibility that ingestion of *A. niger* var. *awamori* would result in a fungal infection.

As corroborative evidence of the safety of chymosin preparation and of the host organism, Genencor submitted the results of several unpublished studies. These included several in vivo studies and one in vitro study on its chymosin enzyme preparation. An unpublished acute oral pathogenicity study conducted in mice using both the host organism and the actual production strain of the host organism demonstrated both were nonpathogenic. An in vitro study was done using several lots of the chymosin enzyme preparation to assess its potential to be clastogenic (i.e., cause chromosomal aberrations) in Chinese hamster ovary cells. The chymosin enzyme preparation was negative for in vitro clastogenicity in this study. In a 13-week in vivo feeding study, no significant adverse effects were observed in the rats fed the chymosin enzyme preparation.

The petitioner also provided two studies that were conducted to test the chymosin enzyme preparation as an irritant. An eye irritation study in rabbits was conducted with the chymosin enzyme preparation. The preparation was an apparent mild irritant; however, all ocular irritation of the treated eyes subsided by 24 hours after treatment. Such a result is not unexpected or relevant to ingestion of the chymosin enzyme preparation. A primary dermal irritation study conducted in rabbits demonstrated that the enzyme preparation was not an irritant. Except for being found to be a minimal irritant in the eye irritation study, the results of the two tests were negative.

In another study, the potential of the enzyme preparation to cause delayed contact hypersensitivity was tested in guinea pigs. In this 3-week study, the response of the test animals to the enzyme preparation was no greater than the response to the control material. The authors concluded that the chymosin enzyme preparation did not induce sensitization in guinea pigs under the conditions of the study.

One potential safety concern raised by cloning is whether extraneous DNA (particularly DNA flanking the gene of interest that could encode proteins of unknown safety) may be cloned along with the gene of interest (i.e., that producing prochymosin) and contaminate the enzyme preparation. As a matter of current good manufacturing practice, manufacturers using recombinant DNA technology should ensure that they have not inadvertently cloned extraneous protein-encoding DNA along with the prochymosin gene. Such assurance can come from reviewing the details of the cloning steps, such as the origin and sequence of the DNA fragments used in the cloning, and from full characterization of the final genetic constructs via techniques such as DNA sequencing. Genencor's petition contains information demonstrating that the company conducted these steps.

For example, the *A. niger* var. *awamori* strains that are used by Genencor (Refs. 9 through 11) to produce the chymosin preparation contain marker genes that encode resistance to a clinically useful antibiotic. The agency evaluated whether these genes could be transferred to other microorganisms with which the production strain or its DNA comes into contact, causing proliferation of antibiotic resistance. As previously described, the isolation of the enzyme includes an acid treatment step that results in the destruction of residual cells and the degradation of residual DNA, including marker genes (Ref. 60).

As corroborative evidence that the enzyme preparation does not contain gene-sized DNA fragments or transformable DNA (that is, DNA that a microorganism can take up from its surroundings and functionally incorporate into its own DNA), Genencor submitted data from several unpublished experiments, including a DNA extraction/Southern blot assay and a transformation assay. The results of the DNA extraction/Southern blot assay showed that no plasmid sequences, including the antibiotic resistance marker, were present in the product. In the transformation assay, bacterial cells were mixed with the enzyme preparation under optimized conditions and assayed to see if they had picked up DNA encoding antibiotic resistance. The cells did not become resistant to the

antibiotic. Based on the facts discussed above, FDA concludes that chymosin preparation manufactured in conformity with § 184.1685(a)(3) will not contain DNA encoding resistance to antibiotics at levels that would produce any safety concern.

Moreover, the regulation stipulates that the substance being affirmed as GRAS is one that is produced using a production strain that is nontoxigenic (see § 184.1685(a)(4)). If the cloned DNA were to encode a harmful substance that could render the enzyme preparation unsafe, the production strain would be toxigenic; in such circumstances, the chymosin preparation would not be GRAS under § 184.1685(a)(4). Therefore, the agency finds that there is no basis for concern that the safety of the chymosin preparation will be compromised by contaminating proteins encoded by extraneous uncharacterized DNA cloned along with the prochymosin gene.

Having considered the evidence concerning the production organism and the processing steps to derive the chymosin preparation, FDA concludes that *A. niger* var. *awamori* is safe for use as a source of food-grade chymosin preparations, and that impurities resulting from its use in the production of chymosin preparation will not affect the safety of the chymosin preparation.

IV. Specifications

The agency finds that, because the principal active ingredient of the chymosin preparation and rennet are the same, and because the potential impurities in the chymosin preparation that may originate from the source organism or manufacturing process do not provide any basis for concern about the safe use of the preparation, the current requirements given for chymosin preparations in § 184.1685(b) are adequate for defining the minimum criteria for a food-grade chymosin preparation derived from *A. niger* var. *awamori*.

V. Conclusion

The agency has evaluated all available information and finds, based upon the published and corroborative evidence discussed above, that the principal active ingredient in the chymosin preparation is the same as that in rennet, and that when the preparation is manufactured in accordance with § 184.1685(a)(4), the source organism and manufacturing process will not introduce impurities into the preparation that may render the use of the preparation unsafe. Therefore, the agency concludes, based upon scientific procedures, that the chymosin preparation derived by fermentation from *A. niger* var. *awamori* and described in the regulation set out below is GRAS for use as a replacement for rennet.

VI. Environmental Impact

The agency has carefully considered the potential environmental effects of this action. FDA has concluded that the action will not have a significant impact on the human environment, and that an environmental impact statement is not required. The agency's finding of no significant impact and the evidence supporting that finding, contained in an environmental assessment, may be seen in the Dockets Management Branch (address above) between 9 a.m. and 4 p.m., Monday through Friday.

VII. Economic Effects

FDA has examined the economic implications of the final rule affirming the GRAS status of chymosin enzyme preparation derived from *A. niger* var. *awamori* as a direct human ingredient, as required by Executive Order 12291 and 12612 and the Regulatory Flexibility Act (Pub. L. 96–54). Executive Order 12291 compels agencies to use cost-benefit analysis when making decisions and Executive Order 12612 requires Federal agencies to ensure that Federal solutions, rather than State or local solutions, are necessary. Pub. L. 96–54 requires regulatory relief for small businesses where feasible. The agency finds that this final rule is not a major rule as defined by Executive Order 12291. In accordance with Pub. L. 96–54, FDA has also determined that this final rule will not have a significant adverse impact on a substantial number of small business. Finally, because this regulation applies to food for interstate trade and individual State regulations would hinder interstate trade, FDA finds that there is no substantial federalism issue which would require an analysis under Executive Order 12612.

Because no current activity is prohibited by this final rule, it will not result in any compliance cost to firms. Also, because no increase in the health risks faced by consumers will result from this final rule, no compliance costs will result. Potential benefits include the wider use of this enzyme because of reduced uncertainty concerning its GRAS status and any resources saved by eliminating the need to prepare further petitions to affirm the GRAS status of this enzyme from this source organism.

VIII. 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.

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List of Subjects in 21 CFR Part 184

Food ingredients.

Therefore, under the Federal Food, Drug, and Cosmetic Act and under authority delegated to the Commissioner of Food and Drugs, 21 CFR part 184 is amended as follows:

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

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

Authority: Secs. 201, 402, 409, 701 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 321, 342, 348, 371).

2. Section 184.1685 is amended by adding new paragraph (a)(4) to read as follows:

§ 184.1685 Rennet (animal-derived) and chymosin preparation (fermentation-derived).

(a) * * *

(4) Chymosin preparation is a clear solution containing the active enzyme chymosin (E.C. 3.4.23.4). It is derived, via fermentation, from a nonpathogenic and nontoxigenic strain of *Aspergillus*

niger van Tieghem variety *awamori* (Nakazawa) Al-Musallam (synonym *A. awamori* Nakazawa) containing the prochymosin gene. Chymosin is recovered from the fermentation broth after acid treatment. All materials used in the processing and formulating of chymosin preparation must be either generally recognized as safe (GRAS) or be food additives that have been approved by the Food and Drug Administration for this use.

* * * * *

Dated: April 29, 1993.

Michael R. Taylor,

Deputy Commissioner for Policy.

[FR Doc. 93–10760 Filed 5–6–93; 8:45 am]

BILLING CODE 4160–01–F

DEPARTMENT OF THE INTERIOR

Geological Survey

30 CFR Part 401

RIN 1028–AA03

State Water Research Institute Program

AGENCY: U.S. Geological Survey, Interior.

ACTION: Final rule.

SUMMARY: The U.S. Geological Survey (USGS) is amending the procedures used to evaluate the State Water Research Institutes to implement the changes to the Water Resources Research Act of 1984. This action is intended to reduce costs associated with the evaluation process for both the USGS and the institutes. The USGS is also removing references to obsolete documents, revising and clarifying the requirements for new institutes, revising the requirements for expenditure of unobligated funds, and making other minor changes to bring the regulation in compliance with the amended Act.

EFFECTIVE DATE: June 7, 1993.

FOR FURTHER INFORMATION CONTACT: Allen Ford, Office of External Research, U.S. Geological Survey, Water Resources Division, 424 National Center, 12201 Sunrise Valley Drive, Reston, Virginia 22092, (703) 648–6806.

SUPPLEMENTARY INFORMATION:

Background

The State Water Research Institutes authorized by the Water Resources Research Act of 1984 (Pub. L. 98–242, 98 Stat. 97) and reauthorized by Water Research Institutes Authorization Through Fiscal Year 1994 (Pub. L. 101–397, 104 Stat. 852) support research, education, and information transfer activities. The 54 institutes in the program are administered and periodically evaluated under the provisions of 30 CFR part 401, adopted in May 1985. The reauthorization amended several provisions of the Water Resources Research Act of 1984, and the USGS is accordingly making minor revisions to the rule guiding the administration and evaluation of the institutes.

The former rule guiding the evaluation of the institutes required that five person teams visit each of the 54 institutes at least once every 5 years. The USGS has made minor revisions to the rule pertaining to institute evaluations by amending subpart E of 30 CFR 401.26 which describes the procedures used to evaluate the State Water Research Institutes. The reauthorization amends section 104(e) of the Water Resources Research Act of 1984 to give the Secretary of the Interior more discretion in the evaluation process. This action revises the rule pertaining to institute evaluations such that: The size of the evaluation team is decreased and its composition changed; the evaluation team will visit only those institutes it considers, on the basis of submitted documentation, to be potential candidates for probation; the composition of the evaluation team is changed; the evaluation team will consider only those institute activities funded under section 104 of the Water Resources Research Act of 1984; evaluation criteria not directly related to performance of the institutes is eliminated; the evaluation team is allowed more time to submit a written report of its findings. The changes will: lower the cost of the evaluation process to both the granting agency and the institutes by minimizing the number of institute site visits; permit greater consistency in the evaluation process by using, to the extent possible, only one evaluation team for all institutes; and base the evaluation only on demonstrated performance in the use of section 104 grants.

Section 401.11(a) of the rule requires that, if the full amount of the available grant funds for any fiscal year has not been requested as of the closing date for receipt of applications, any remaining funds shall be made available to the institutes for amended applications. The USGS is revising this section to state that any such remaining funds be made available to support competitively selected research projects under the terms of section 104(g) of the Act, as required by the reauthorization.

The USGS is amending section 401.11(g) to state that Federal funds received by the institutes shall be matched on a basis of no less than two non-Federal dollars for each Federal dollar, as required by the reauthorization, unless the grant is exempt under the provisions of 48 U.S.C. 1469a(d) as amended.

The USGS is amending section 401.12(c) to remove references to obsolete documents and add references to new documents guiding the institutes' administration of the grants received under section 104 of the Act.

Response to Public Comment

Two comments were received in response to the proposed rule as published in the **Federal Register** (57 FR 59941) on December 17, 1992. A discussion of the comments follows:

Comment: The existing rule (30 CFR 401.3) defines "State" to include the Trust Territory of the Pacific Islands. Pub. L. 101–397 amended the Water Resources Research Act of 1984 by changing "Trust Territory of the Pacific Islands" to "Federated States of Micronesia," and the language of the rule should be changed accordingly.

Response: The language of section 401.3 has been modified to adopt the suggested change.

Comment: The proposed revision to section 401.11(g) states that the Federal funds are to be matched on a basis of no less than two non-Federal dollars for each Federal dollar. Guam, the Federated States of Micronesia, and the Virgin Islands are not required to match the Federal funds received under this grant.

Response: The language of section 401.11(g) has been modified to recognize that, under the provisions of 48 U.S.C. 1469a(d) as amended, the matching requirement may be waived for applicants from specified Insular Areas.

Required Analyses

The Department of the Interior has determined that this document is not a major rule under Executive Order 12291 and certifies this document will not have a significant economic effect on a substantial number of small entities under the Regulatory Flexibility Act (5 U.S.C. 601 *et seq.*). This action will promote efficiency and economy by reducing costs for both the Government and the institutes. Therefore, it will not adversely affect the economy of the Nation or any small entity.

Environmental Effects

This action will have no potential for significant environmental impact and is categorically excluded from the requirements for compliance with the