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Guidance for Industry

Enzyme Preparations: Recommendations for Submission of Chemical and Technological Data for Food Additive Petitions and GRAS Notices

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Office of Food Additive Safety
Division of Biotechnology and GRAS Notice Review, HFS-255
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, MD 20740
(Tel) 301-436-1200
<http://www.fda.gov/FoodGuidances>

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U.S. Department of Health and Human Services
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This guidance represents the Food and Drug Administration's current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the telephone number listed on the title page of this guidance.

I. Introduction

This document describes chemical and technological data that the FDA's Office of Food Additive Safety (OFAS) in the Center for Food Safety and Applied Nutrition considers when evaluating food additive petitions and GRAS notices for enzyme preparations.

This guidance does not discuss data and information relevant to microbiological, toxicological and environmental considerations. It also does not consider the safety of substances used to immobilize enzymes. Such substances should be approved through either a food additive petition or a food contact notification. This guidance will be updated as needed to reflect new developments in the manufacture and use of enzyme preparations.

This document revises and supersedes the guidance entitled "Enzyme Preparations: Chemistry Recommendations for Food Additive and GRAS Affirmation Petitions" dated January 1993.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not necessarily required.

II. Background

Enzyme preparations used in food processing contain an active enzyme that is responsible for the intended technical effect in food. In some instances, enzyme preparations may contain a blend of

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two or more active enzymes. Enzymes present in enzyme preparations may be derived from a variety of biological sources, such as plants, animal tissues, or microorganisms. Enzymes obtained from these sources are formulated with intentionally added ingredients, such as diluents, preservatives, stabilizers or other substances suitable for use in food. Enzyme preparations may also contain constituents derived from the enzyme source and the manufacturing process, for example, the residues of a fermentation medium used for growing the microorganism that produces the enzyme. Enzyme preparations are used in food processing at very low levels and often are removed from the final food or inactivated. For example, enzymes used in processing starch into glucose or high fructose corn syrup are removed during purification of the syrup, while enzymes used in baked goods are inactivated by high temperatures during baking.

Enzyme preparations can be regulated as secondary direct food additives under [Title 21 of the Code of Federal Regulations, Part 173 \(21 CFR 173\)](#). The regulatory status of food additives, including secondary food additives, is established through the petition process. According to Section 409(b)(1) of the Federal Food, Drug, and Cosmetic Act (the Act) [21 U.S.C. 348(b)(1)], anyone may file a petition proposing the issuance of a regulation. Section 409(b)(2) of the Act [21 U.S.C. 348(b)(2)] prescribes the statutory requirements for food additive petitions. The requirements for food additive petitions are discussed in greater detail in 21 CFR 171.1.

Section 201(s) of the Act [21 U.S.C. 321(s)] exempts the use(s) of a substance that is generally recognized as safe (GRAS) from the definition of a food additive. A substance can be determined to be GRAS under the intended conditions of use if there is evidence of its safety (the “technical element” of the GRAS standard) and a basis to conclude that this evidence is generally known and accepted by qualified experts. Title 21 of the CFR contains regulations for food ingredients that are either listed as GRAS or affirmed as GRAS through GRAS affirmation petitions. In the past, FDA reviewed GRAS affirmation petitions for enzyme preparations. A successful review of a GRAS affirmation petition resulted in a regulation in 21 CFR, Part 184. The GRAS affirmation process has been replaced by a voluntary notification program under the agency’s proposed regulation (Proposed 21 CFR 170.36 (62 FR 18938; April 17 1997; Substances Generally Recognized as Safe (GRAS))).

III. Discussion

As noted above, an enzyme preparation may be either regulated as a secondary direct food additive through a premarket approval process or determined to be GRAS. In either case, the safety determination is limited to the intended conditions of use of an enzyme preparation.

A. Petitions for Enzyme Preparations

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Section 409(b)(2) of the Act describes the statutory requirements for food additive petitions, which encompass five general areas of information:

1. The identity of the additive.
2. The proposed use of the additive.
3. The intended technical effect of the additive.
4. A method of analysis for the additive in food.
5. Full reports of all safety investigations with respect to the additive.

[21 CFR 171.1\(c\)](#) describes in greater detail the data to be provided in food additive petitions, including administrative information. Food additive petitions for enzyme preparations should contain data and information pertinent to the above five basic areas. However, the specific information within each of these areas should be compatible with the nature of enzyme preparations which, in addition to the enzyme(s) of interest, contain intentionally added ingredients and may also contain unidentified constituents derived from the enzyme source and manufacturing process. The experimental data related to chemistry issues should be described in a clear and concise manner and supported with raw data and appropriate analytical methods.

As described in [21 CFR 171.1\(h\)](#), certain data and information contained in food additive petitions are available for public disclosure, while other data and information are not. Questions in this regard should be directed to OFAS.

B. GRAS Notices for Enzyme Preparations

General recognition of safety may be based either on scientific procedures (21 CFR 170.30(b)) or, for substances used in food prior to January 1 1958, on experience based on common use in food (21 CFR 170.30(c)). A GRAS determination through scientific procedures requires common knowledge about the substance and its safety under the intended conditions of use throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food. This determination can be made by qualified experts outside of government.

FDA has proposed a voluntary notification program whereby a person may notify FDA of its determination that a substance is GRAS (Proposed 21 CFR 170.36 (62 FR 18938; April 17, 1997; Substances Generally Recognized as Safe (GRAS)) (available at: [Federal Register Notice – the GRAS Proposal](#) or the [GPO website](#); search for 62 FR 18938) If the data and information provided in the GRAS notice do not raise safety concerns about the use of the substance at the time of the review, FDA issues a letter to the notifier stating that the agency has no questions regarding the notifier’s conclusion that the substance is GRAS under the intended conditions of use.

In recent years, numerous enzyme preparations intended for use in food processing were evaluated on the basis of scientific procedures through the GRAS notification program. GRAS

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notices for enzyme preparations contain a GRAS exemption claim, technical information showing that there is a reasonable certainty in the minds of competent scientists that the enzyme preparation is safe under the intended conditions of use, and evidence that this information is generally known and accepted by the scientific community. The technical information may include but is not limited to the identity, method of manufacture, specifications, use levels, dietary exposure, and toxicological studies.

The technical information in a GRAS notice is essentially the same as in a food additive petition. However, FDA encourages the notifiers to submit only a summary of data and information in GRAS notices and retain the detailed records and raw data for FDA review upon request. FDA discourages including proprietary information in GRAS notices, as the notices are available for public disclosure in compliance with the Freedom of Information Act. If a GRAS notice contains proprietary information, the notifier should clearly designate the information as confidential. FDA will review the designated information, determine whether that information is exempt from public disclosure under 21 CFR part 20, and release or protect the information in accordance with that determination.

A GRAS notice should also contain a discussion of any other generally available and accepted data and information that the notifier relied upon to support the GRAS determination of an enzyme preparation under the intended conditions of use, as well as any information that may appear to be inconsistent with the GRAS determination. Not all GRAS notices will need to be supported by the same quantity and quality of data. Questions regarding the tailoring of the data package for specific cases may be discussed with agency personnel prior to submitting a GRAS notice. The notifiers may also review FDA's information on the [GRAS notification program](#).

IV. Recommendations for Petition and GRAS Notice Preparation

A. Identity

In the "Identity" section of a petition or GRAS notice, the enzyme preparation should be characterized as completely as possible. The characterization should include the identity and characterization of the enzyme, enzyme source, and the final enzyme preparation. The items to be considered under "Identity" are given below.

1. Identity of the enzyme

- Accepted name, systematic name, and the Enzyme Commission (EC) number according to the recommendations of the International Union of Biochemistry and Molecular Biology.²
- Other name(s).
- Chemical Abstract Service Registry Number (CAS No.), if available.

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- Enzymatic activity, substrate specificity, and the reaction catalyzed.
- Other properties, such as specific activity, molecular weight, amino acid sequence, and stability and activity as a function of temperature and pH, if available or necessary to support the enzyme identity and its intended uses.
- Structural modifications introduced by chemical or genetic methods that affect enzyme performance under the intended conditions of use.
- The characteristics of the enzyme that may be useful in assessing if the enzyme is likely to be a food allergen. The information relevant to the assessment of potential allergenicity should be consistent with current views of the scientific community and may include the considerations regarding the enzyme source, comparison of the amino acid sequence of the enzyme to the amino acid sequences of known allergens, susceptibility of the enzyme to pepsin digestion, and other relevant information, if available.

2. Characterization of the enzyme source

Enzymes currently used in food processing are derived from animal tissues, plant material, and microorganisms. These sources should comply with the principles outlined in the monograph “Enzyme Preparations” (section “Other Requirements”) of the 6th (2008) or current edition of the Food Chemicals Codex (FCC).³ Animal tissues used to produce enzymes should comply with the applicable U.S. meat inspection requirements and should be handled in accordance with good hygienic practices. Plant material used to produce enzymes and culture media used to grow microorganisms should consist of components that leave no residues harmful to health in the finished food under normal conditions of use. The fermentation process should be conducted under controlled conditions to prevent contamination with microorganisms that could be the source of toxic and other undesirable substances.

Microorganisms used in the production of enzymes should be taxonomically identified and shown to be nonpathogenic and nontoxic. However, certain strains of microorganisms normally considered to be nontoxic may be capable of producing toxins when cultured under conditions that are conducive to toxin synthesis. When such microorganisms are used as sources of enzymes, the fermentation conditions should be adjusted to prevent toxin synthesis and appropriate tests should be conducted to assure that the final enzyme preparations do not contain toxins at unsafe levels. Alternatively, such microorganisms may be genetically modified to inactivate biochemical pathways involved in toxin synthesis. This can be accomplished either by classical mutagenesis or genetic engineering. All the information relevant to the identity and safety of the microorganisms used as sources of enzymes should be described, including current and previous uses in food or in the production of food ingredients, if applicable.

Additional information should be provided for enzymes derived from genetically modified microorganisms (GMMs).⁴ Such microorganisms should be thoroughly characterized with respect to any introduced DNA. The source(s) of the introduced DNA including the gene(s) encoding the enzyme(s) of interest, any other genes (e.g., genes encoding selectable markers), and regulatory DNA sequences necessary for gene expression should be identified. The enzyme-encoding genes can be derived from known organisms, unidentified organisms sampled from the

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environment, or generated from a pool of genes from various sources via molecular evolution also known as gene shuffling. The enzyme-encoding genes can also be synthesized or modified by traditional or site-specific mutagenesis to adapt the enzyme properties to the specific food application conditions or to enhance the enzyme production.

The host microorganisms can also be modified by inactivating or deleting certain endogenous genes, for example, to prevent the synthesis of potentially harmful secondary metabolites (e.g., mycotoxins) or to minimize the production of other enzymes that may interfere with the production of the enzyme of interest or its function in food processing. All approaches and steps involved in the production of enzymes from GMMs should be described.

3. Composition of the enzyme preparation

Commercial enzyme preparations that are used in food processing typically contain an enzyme, which catalyzes the chemical reaction that is responsible for its technical effect, as well as substances used as stabilizers, preservatives or diluents. Enzyme preparations may also contain constituents derived from the production organism and manufacturing process. To characterize the enzyme preparation the following information should be provided:

- Identities and levels of diluents, stabilizers, preservatives, and any other substances used in the enzyme formulation.
- Information on secondary enzymes derived from the production organism(s) that may be present in the enzyme preparation.
- Information on the residues of other metabolites derived from the production organism(s) and substances used in enzyme isolation and purification.
- The content of total organic solids (TOS) for both the commercial enzyme preparation and the enzyme batch used in toxicology studies. TOS is the sum of all organic compounds present in the enzyme preparation derived from the enzyme source and manufacturing process.⁵ TOS is calculated as follows:

$$\text{TOS (\%)} = 100 - A - W - D$$

Where:

A = % ash

W = % water

D = % diluents and other formulation ingredients

B. Manufacturing Process

Regardless of the source material, enzyme preparations should be produced in accordance with the current good manufacturing practice (cGMP). For enzyme preparations obtained from animal

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or plant materials including tissue culture, the source materials should be characterized and the procedures for enzyme isolation and purification should be described.

For enzyme preparations obtained from microorganisms, the fermentation process should be described, including all steps and controls necessary to maintain the proper growth conditions and purity and genetic stability of the culture. The isolation of the enzyme from the cellular material or from the fermentation broth (depending on whether the enzyme is intracellular or secreted) should be described, including all chemical and physical treatments and quality controls. All materials used in the fermentation and subsequent downstream processing (including antifoaming and flocculating agents, if used) should be identified and shown to be suitable for use in food processing.

C. Specifications for Identity and Purity

Enzyme preparations should conform to the purity specifications for enzyme preparations provided in the monograph “Enzyme Preparations” of the 6th (2008) or current edition of the Food Chemicals Codex (FCC). Petitioners and notifiers should rely on the analysis of several batches of the enzyme preparation to establish conformance with the specifications. The standard analytical methods (e.g., those described in FCC) used in the specifications should be referenced, while the non-standard methods, such as those elaborated by the manufacturer, should be validated and described and the unit of activity should be defined.

Enzyme preparations obtained from microbial sources should not contain antibiotics, toxins (e.g., protein toxins or mycotoxins) or transformable DNA coding for protein toxins or proteins that inactivate therapeutic antibiotics. If the absence of these substances cannot be established on the basis of the characteristics of the production strain, appropriate tests should be performed to show that the enzyme preparation does not contain these substances at biologically significant levels.

D. Intended Technical Effects and Use

All foods, or groups of foods, in which the enzyme preparation is used or intended to be used should be specified and the technical effects of the enzyme should be described. The use level, or range of levels, should be provided on the basis of TOS for each food or food group and expressed in metric units (e.g., in milligrams of TOS per kilogram of food).

The fate of the enzyme preparation in food should be described. The enzyme preparation may be carried over to food or be removed from food prior to consumption, or the enzyme may be

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inactivated during food preparation (e.g., baking) or remain active in the final food. If the enzyme preparation is removed from food, the residual level of the enzyme preparation TOS in food "as consumed" should be estimated, if possible.

The reaction products formed in food as a result of enzyme activity should be identified and their safety should be addressed.

E. Intake Estimate

A food additive petition or GRAS notice should contain an estimate of the dietary exposure to the enzyme preparation, i.e., an estimated daily intake (EDI). The EDI should be based on food intake and the level of enzyme TOS in food. If the actual TOS level in the final food is not available, the EDI may be calculated on the basis of the use level expressed as TOS. The sources of food consumption data should be referenced and briefly described.

V. Additional Information

A. Enzyme Preparations Used in Meat, Poultry, and Egg Products

FDA and FSIS developed a Memorandum of Understanding (MOU),⁶ which provides for a simultaneous review of substances intended for use in or on meat and poultry products that are subjects of food and color additive petitions and GRAS notices. If an enzyme preparation is intended for use in or on meat and poultry products, FDA will consult with the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture consistent with this MOU.

According to the MOU, FDA evaluates information related to the safety of the substance under the intended conditions of use, while FSIS evaluates data that support the suitability of the use of the substance. Therefore, a food additive petition or a GRAS notice for an enzyme preparation to be used in or on meat and poultry products should contain information pertinent to both, the safety and suitability of the use of the enzyme preparation. Regarding the suitability of the use of an enzyme preparation, data should be provided showing that the enzyme preparation will be used at the lowest level necessary to achieve the intended technical effect under the proposed conditions of use. These data should be provided for each meat and poultry category in which the use of an enzyme preparation is intended. FDA will provide this information to FSIS and coordinate a joint review consistent with the MOU.

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FSIS also evaluates the suitability of substances in the formulation of egg products as defined in 9 CFR 590.5. Therefore, a food additive petition or a GRAS notice submitted to FDA for an enzyme preparation intended for use in egg products regulated by FSIS should contain information on the suitability of the use of the enzyme preparation in egg products.

B. Enzyme Preparations Containing Allergenic Ingredients

A food additive petition or a GRAS notice for an enzyme preparation should contain information on the presence of proteins derived from major food allergens that fall under the provisions of the [Food Allergen Labeling and Consumer Protection Act of 2004](#) (FALCPA). FALCPA requires that the labels of all FDA-regulated foods that are or contain a major food allergen must disclose the food source name of the allergen, unless the ingredient has been exempted from labeling through either a petition or notification process. According to FALCPA, a major food allergen is defined as one of the following foods and ingredients that contain protein from them: milk, egg, peanuts, tree nuts, soybeans, wheat, fish, and Crustacean shellfish. The food source names of the major food allergens are milk, egg, peanuts, the specific type of tree nut (e.g., almonds), soybeans, wheat, the specific species of fish (e.g., flounder) and the specific species of Crustacean shellfish (e.g., crab).

If an enzyme preparation contains proteins derived from a major food allergen and is used in foods that normally do not contain this allergen, such an enzyme preparation may fall under the provisions of FALCPA unless it is granted an exemption from allergen labeling as a result of a petition or a notification. For example, such a situation may occur if an enzyme preparation has been formulated with wheat flour and is intended for use in foods that normally do not contain wheat protein. More information on [food allergen labeling requirements](#) may be found in the FDA Guidance documents for Food Labeling and Nutrition.⁷

¹ This guidance has been prepared by the Division of Biotechnology and GRAS Notice Review in the Center for Food Safety and Applied Nutrition at the U.S. Food and Drug Administration.

² <http://www.chem.qmul.ac.uk/iubmb/enzyme/search.html>

³ Food Chemicals Codex, 6th Edition (2008). The United States Pharmacopeial Convention. Available in print or online at: <http://online.foodchemicalscodex.org>

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⁴ The term “genetically modified microorganism” (GMM) means for the purpose of this document a microorganism that has been modified by modern recombinant DNA techniques. However, the scientific meaning of this term is much broader and encompasses any intentional genetic modification accomplished by either traditional or rDNA methods.

⁵ NRC/NAS, 1981. The 1978 Enzyme Survey Summarized Data. National Research Council/National Academy of Sciences. Washington, D.C., 1981. U.S. Department of Commerce. National Technical Information Service.

⁶ Memorandum of Understanding Between The Food Safety and Inspection Service, United States Department of Agriculture, and The Food and Drug Administration, United States Department of Health and Human Services.

http://origin-www.fsis.usda.gov/Regulations_&_Policies/Labeling_FDA_MOU/index.asp

<http://www.fda.gov/AboutFDA/PartnershipsCollaborations/MemorandaofUnderstandingMOUs/DomesticMOUs/ucm112604.htm>

⁷<http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/FoodLabelingNutrition/ucm059116.htm>