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Guidelines for the Risk Assessment of Additives (Enzymes) in Foods

Chapter 1: General Provisions

Article 1: Background

The Food Safety Commission of Japan (FSCJ) endeavors to establish guidelines for the assessment of food-related hazards on human health (hereinafter referred to as “the risk assessment”), based on “Basic Matters in Article 21 paragraph (1) of the Food Safety Basic Act” (the Cabinet Decision, June 29, 2012).

Accordingly, the following guidelines have been established: “Standards for the Safety Assessment of Genetically Modified Foods (Seed Plants)” (January 29, 2004), “Stance on Assessment of the Effect of Foods on Human Health Regarding Official Specifications of General Fertilizers” (March 18, 2004), “Standards for Safety Assessment of Food Additives Produced Using Genetically Modified Microorganisms” (March 25, 2004), “Stance on Safety Assessment of Genetically Modified Feeds and Feed Additives” (May 6, 2004), “Assessment Guideline for the Effect of Foods on Human Health Regarding Antimicrobial-Resistant Bacteria Selected by Antimicrobial Use in Food Animals” (September 30, 2004), “Standards for Safety Assessment of Genetically Modified Foods (Microorganisms)” (June 26, 2008), “Guideline for Assessment of the Effect of Foods on Human Health Regarding Food Additives” (May 27, 2010) (hereinafter referred to as “Guideline for Assessment of Additives”)¹⁾, and Guidelines for the Assessment of Flavoring Substances in Foods on Health (May 17, 2016).

Guidelines for the risk assessment are essential for ensuring the scientific validity and fairness of assessments as well as for clarifying the data required for application, keeping the transparency of assessment both within and outside Japan.

While safety assessments of enzymes that are used as additives have been conducted according to the Guideline for Assessment of Additives, processing aids including enzymes^{*1} are not

^{*1} The term “Processing aid” in these guidelines designates the substance that meets one of the following conditions, among food additives used in food processing:

- 1) An additive that is removed from the food before final packaging.
- 2) An additive that is converted to a naturally contained component in food, and the amount of the component does not significantly increase the amount of the natural component.

necessarily classified as food additives worldwide. In addition, the risk assessment of enzymes needs to include a safety evaluation of the source organisms, allergenicity^{※2} of the enzyme, and degradability in the gastro-intestinal tract.

Recently, a study entitled “Study on the Procedure for the Risk Assessment of Food Additives for Fortification and Processing Aids in Japan” (Principal Investigator: Takashi Umemura, National Institute of Health Science) was conducted with the support of a FSCJ grant for Research and Survey Program. The study summarized the draft Guidelines for the risk assessment of processing aids⁵⁾, taking into account the results of the previously conducted risk assessments of enzymes^{2,3)}, the survey report⁴⁾ on approaches for the risk assessment of processing aids employed in the Joint FAO/WHO Expert Committee on Food Additives (JECFA^{※3)}, U.S. Food and Drug Administration (FDA), European Food Safety Authority (EFSA), and Food Standards Australia New Zealand (FSANZ).

FSCJ finalized the new guidelines for the risk assessment of enzymes based on the research report by Umemura’s group. Thus, the risk assessment of enzymes is conducted according to these guidelines from now on.

The guidelines would be revised after reviewing their provisions, taking into account international trends of assessment guidelines and new scientific findings both within and outside Japan, if needed.

Article 2: Purpose

The purpose of the guidelines is to establish the guiding principle of risk assessment on enzymes that are classified as additives and to define the scope of the required documents. The term "additives" as used in these guidelines shall mean substances that are used by being added, mixed, or infiltrated into food or by other methods in the process of producing food or for the purpose of processing or preserving food, as provided in Article 4, paragraph (2) of the Food Sanitation Act (Act No. 233, 1947). The risk assessment is to be conducted for the cases where the Minister of Health, Labour and Welfare (MHLW) specifies as having no risk to human health as provided in Article 10 of the Food Sanitation Act, or where MHLW intends to establish standards or specifications in accordance with the provisions of Article 11, paragraph (1) of the Act, or where MHLW hears the Commission’s opinions if they are recognized as necessary for formulating policies to ensure food safety in accordance with Article 24, paragraph (3) of the Food Safety Basic Act (Act No.48, 2003).

Article 3: Approach for the Risk Assessment of Enzymes

This article describes the approach for the risk assessment of enzymes. The details and points to be considered are described in Chapter 2.

3) An additive that is found only in trace amounts in the final food product and has no effect on the food.

※2 The term “allergenicity” specifies, hereinafter, both the allergy-inducing and sensitizing effects, including the inducibility of gluten-induced enteritis.

※3 Abbreviations used in text are explained in the appendix.

1. Amount of enzymes

Amount of enzymes shall be expressed in principle as the total organic solid (TOS)^{※4}, because the weight of enzymes depends on how much concentrated.

2. Procedure of the safety evaluation

First, safety of the source organisms and safety of enzymes of the target in the assessment (hereinafter referred to as “the target enzyme”) shall be evaluated. Safety of mixed impurities may also be evaluated if needed.

In the safety evaluation of enzymes, “Points relevant to degradability in the gastro-intestinal tract” designated in Article 2, paragraph (2) of Chapter 2 would be evaluated. When points 1) to 5) are all considered to be fulfilled through the evaluation, toxicity of the enzyme such as 90-day repeated dose toxicity in rodents, genotoxicity, and allergenicity shall be evaluated^{※5}.

3. Determination of NOAEL

When a no-observed-adverse-effect level (NOAEL) of the target enzyme is determined in a toxicity study, it should be examined whether the appropriate doses are assigned or not. In particular, the maximum dose in a toxicity study shall correspond to a dose at which some toxic effects are observed, and the minimum dose shall correspond to a dose at which no toxic effect is observed. Moreover, each dose examined in a study should be selected so as to provide a dose-response relationship. In the case of a study with dietary administration, care should be taken to prevent nutritional disorder. Generally, a dosage of a feed containing the target enzyme above 5% (W/W) is unnecessary. If no adverse effect is observed in a study with gavages using a technically feasible maximum dose or 1,000 mg/kg body weight, administration of the higher dose is unnecessary.

When two or more animal studies are conducted with different animal species, NOAEL can be determined in each study. In such a case, the lowest value of those NOAELs shall be determined as the NOAEL for the conclusive evaluation of the target enzyme. However, if one particular study is apparently more appropriate in the experimental design or in the results than other studies, or the studies are carried out for different terms, a longer and more appropriate study shall be considered with a special emphasis in determining the NOAEL for the conclusive evaluation. In addition, when the metabolic or pharmacodynamic data are available for the target enzyme, a value of the NOAEL for the conclusive evaluation may be determined based on the toxicity study conducted in animal species that shows toxic responses closest to those of humans.

4. The Risk assessment

The risk assessment of the target enzyme shall be conducted comprehensively based on the safety evaluation of the source organisms, degradability in the gastro-intestinal tract, toxicity data, and the estimated daily intake (EDI) of the target enzyme.

If the target enzyme fulfills points 1) to 5) in the “Points relevant to the degradability in the gastro-intestinal tract” designated in Article 2, paragraph (2) of Chapter 2, 90-day repeated dose toxicity in rodents, genotoxicity, and allergenicity shall be evaluated and the NOAEL shall be

^{※4} In use of TOS, % TOS is calculated by the following equation:

% TOS = 100 – (A+W+D), where A: % ash, W: % water, D: % diluents and/or other formulation ingredients

^{※5} If there are other findings on the safety, these findings would be evaluated in addition.

determined. Then, the margin of exposure (MOE) shall be evaluated comparing the NOAEL with the EDI.

Meanwhile, a daily intake of the target enzyme is estimated on the assumption that whole amount is transferred into the final product and consumed. Therefore, if the enzyme is denatured/inactivated or degraded/removed from the final product or during the food production, the daily intake may be overestimated. Moreover, the enzyme often poses no toxic effect, even at the maximum dose in the repeated toxicity study. Thus, the maximum dose is often determined to be the NOAEL. Hence, the MOE shall be evaluated comprehensively, taking the above-mentioned fact into consideration.

5. Others

- 1) If data suggesting potential risks are available, the risk assessment in pregnant women, fetuses, infants, children, and the elderly shall be conducted as necessary.
- 2) It is desirable to consider the use of *in-vitro* studies that are widely conducted in other fields such as drug development and recommended to be employed in the risk assessment of enzymes, if needed. For example, the *in-vitro* data obtained using human cultured cells or using human metabolic enzymes may be extrapolated to humans in case the adverse effects from the metabolites are present in animal studies.
- 3) Studies using methods that are scarcely employed currently in JECFA and in FSCJ, such as studies in genetically modified animals, have to be considered carefully.
- 4) Points relevant to interactions of the target enzyme with pharmaceuticals shall be studied when there is any information that may suggest risks from such an interaction.
- 5) Safety of combined intake of multiple enzymes is considered to be secured substantially by sufficiently conducting the safety assessment of individual enzymes based on the report from “Survey of information on combined effects of multiple food additives,” in Comprehensive Survey of Securing Food Safety, 2006. When there is any finding of risk from combined intake of multiple enzymes, however, safety shall be evaluated as necessary.

Article 4: Approach for the Documents Required

1. Documents required

The following documents are required for the risk assessment of the target enzyme. Details and points to be considered regarding each item are described in “Detailed Exposition” in Chapter 2. As a general rule, practical procedures of each study are recommended to follow the test guidelines that are approved internationally, such as those of the Organisation for Economic Co-operation and Development (OECD).

- 1) Outline of the target enzyme
- 2) Information concerning safety of the target enzyme (refer also to “Chapter 1, Article 3: Approach for the Risk Assessment of Enzymes”)
- 3) Estimation of daily intake

2. Points to be considered in the amendment of standards for use or specifications

1) The following points shall be considered in the amendment of standards for use.

a) For the amendment of standards for use of the target enzyme of which risk assessment by FSCJ is already completed, applicants shall submit documents concerning the estimation of the daily intake of the enzyme considering requested addition of foods in which the enzyme is to be used or considering changes in the amount of use of the enzyme. Even in this case, applicants shall also submit documents regarding new toxicological findings, if any.

b) When the risk assessment of the target enzymes is not yet completed by FSCJ, applicants in principle shall submit documents required for the evaluation.

2) For the amendment of specifications, applicants need to demonstrate the validity of amended specifications and describe that the amendment requested would not raise safety issues surrounding the target enzyme.

3. Reliability of the documents

Applicants are to submit documents relevant to the evaluation on their own responsibility, and the applicants should also secure reliability of the provided information. As documents relevant to the evaluation, applicants in principle shall provide the following: data of studies conducted using a method with secured reliability at an adequately administered test facility such as a GLP-conforming test facility, risk assessment reports from the international organizations, and scientifically reliable articles. As for data suggesting safety concern of the enzyme, however, applicants shall provide the data irrespective of the reliability because such information may be necessary for evaluating the enzyme.

4. Autopsy and histopathological evaluation

FSCJ recommends performing autopsy and histopathological evaluation by experienced experts.

5. Raw data and specimens

Applicants shall keep existing raw data and specimens from animal studies used for the request until the evaluation becomes complete; shall be ready to provide them as necessary.

6. Additional documents for evaluation

Evaluation in principle shall be conducted using the documents submitted by the applicant. If the documents are considered insufficient for evaluation, the applicant shall be asked for additional documents.

Article 5: Re-evaluation

Potential harmful effects of the enzyme need to be observed continuously even in the case of an approved enzyme. If potential harmful effects of such an enzyme are suggested by advances in science and technology, a re-evaluation of the enzyme should be conducted.

When important data get newly acquired that cause doubts about the safety of enzymes evaluated in the past, a re-evaluation of the enzyme should be conducted immediately.

Chapter 2: Detailed Exposition

Article 1: Outline of the Target Enzyme

The following information on the target enzyme is required.

1. Name and usage

2. Origin or history of discovery

3. Usage in other countries

4. Risk assessments by international organizations and other organizations

5. Physicochemical properties

Chemical name (generic names in Japanese and English, EC (Enzyme Commission) number, and CAS number), source, manufacturing method, constituent (including weight, isoelectric point, and amino acid sequence), property, usage, stability, draft specifications, etc.

6. Draft standards for use

1) When setting the standards for use is considered necessary for specifying subject foods for use and the amount of use, etc. based on the comprehensive evaluation of safety and efficacy of the enzyme, rationales for setting the standards for use need to be clarified.

2) When setting the standards for use is considered unnecessary, rationales for the consideration need to be clarified.

7. Others (information useful for the risk assessment)

Article 2: Information relevant to safety

1. Safety of the source organisms

Safety of the source organisms (animals, plants, microorganisms, etc.) shall be evaluated based on following points.

1) Points relevant to pathogenicity and potential production of harmful substances^{※6}.

a) Pathogenicity

The producer species (or the producer strain in case that the source is a microorganism) for the target enzyme should be proven to be non-pathogenic, in principle, confirming essential points such as the use experience in the production of additives or food experience and taking into consideration the findings on the pathogenicity of the species closely related to the producer species (or the closely related strain in case that the source is a microorganism. The same applies hereinafter in Article 2, paragraph (1) of Chapter 2, “Safety of the source organisms”).

b) Potential production of harmful substances

^{※6} Harmful substances are the substances, including metabolites that pose harmful effects. The same applies hereinafter.

Potential production of harmful substances by the producer species as well as by the closely related species for the target enzyme has not been known in food experience and the knowledge from literature etc. When the potential production of harmful substances cannot be excluded, the harmful substances are to be undetected in the producer species, the enzyme itself, and the enzyme products^{※7}.

2) Points relevant to parasitism and fixing property

Source organisms do not parasitize or fix themselves on humans or other organisms. If parasitism or fixing property of the producer organisms cannot be excluded, there needs to be a rationale to conclude that production using the source organisms is of no safety concern, taking into consideration whether or not the relevant organisms are harmful to humans or other organisms.

3) Points relevant to pathogenic exogenous factors (Viruses etc.)

Source organisms are to be uncontaminated with pathogenic exogenous factors such as viruses.

2. Points relevant to degradability of the enzyme in the gastro-intestinal tract

Degradability of the enzyme in the gastro-intestinal tract shall be evaluated examining the following points.

- 1) The enzyme is readily broken down in the gastro-intestinal tract. Degradability of the enzyme needs to be examined, in principle, by the test described in 4 “Examination of the enzyme for the degradability in the gastro-intestinal tract and the allergenicity,” ensuring that the enzyme is broken down to the mass with no allergenicity concern.
- 2) The main factors concerned in this break down, such as pH and enzymes, need to be clarified.
- 3) The enzyme, when given in moderate amounts and under conditions that will prevail if used as a food additive, or its degradation products are absorbed to the same extent as the food materials to which it gives rise, and do not interfere with the absorption of other nutrients.
- 4) When food utilizing the enzyme is ingested, the enzyme and its degradation products do not cause overloading of the most important food components in the enzyme.
- 5) The enzyme, not being degraded in the gastro-intestinal tract, is not excreted in stools in significant amounts of unbroken or partly broken down form. The unbroken or partly broken down form does not accumulate in the body tissues.

3. Enzyme toxicity

1) 90-day repeated dose toxicity study in rodents

Studies are to follow the points described in 2(1) of Article 2 of Chapter 2 “Subchronic toxicity study and chronic toxicity study” of the Guideline for Assessment of Additives.

2) Genotoxicity study

Studies are to follow the points described in 2(6) of Article 2 of Chapter 2 “Genotoxicity study” of the Guideline for Assessment of Additives.

^{※7}As for substances that are potentially harmful upon the large intake, it has to be ascertained that the substance is undetected in the range in which the substance causes adverse health effects.

3) Allergenicity

a) Potential concern of allergenicity shall be comprehensively evaluated based on the following points. Some of the points may be omitted if there are rationales.

i) Points relevant to allergenicity of the source organisms.

Characteristics relevant to allergenicity of the source organisms are to be clarified.

ii) Points relevant to allergenicity of the enzyme.

Characteristics relevant to allergenicity of the enzyme are to be clarified.

iii) Points relevant to changes in physicochemical properties of the enzyme.

Degradation of the enzyme to the form with no concern of allergenicity shall be shown through the changes in its molecular weight, enzymatic activity, immunoreactivity, etc. Degradation of the enzyme needs to be examined, in principle, by the methods described in 4 “Examination of the enzyme for the degradability in the gastro-intestinal tract and the allergenicity,” confirming that the enzyme is broken down to the mass with no allergenicity concern^{※8, 6)}.

iv) Points relevant to the structural homology of the enzyme with a known allergen (a protein which shows allergenicity, including a protein related to gluten induced enteritis. Hereinafter, “allergen etc.” is used.).

It should be clarified that the enzyme does not have similarities in the amino acid sequences with any known allergen compared with the primary structures. To reveal the amino acid sequence that may represent the epitope, homology survey on amino acid sequences and other possible measures need to be conducted. In that case, the name of the allergen database used, survey criteria, survey method, and the results of the survey are to be provided. The comparison of amino acid sequence with known allergens, in principle, is to be conducted in silico detecting contiguous eight amino acid sequences^{※9, ※10} and searching for matches with 35% or greater identity over a length of 80 amino acids^{※11, 7)}.

b) When potential concern of allergenicity, such as cross-reactivity with known allergens, cannot be excluded through the evaluation described in a), IgE-binding activity of the enzyme shall be examined.

A serum of allergic subjects is to be selected based on the following criteria. If an adequate serum cannot be acquired with the criteria i) or ii) (see below), a serum with specific IgE antibody of high titer against species closely related to the source organisms shall be selected. If an adequate serum still cannot be acquired, a serum with specific IgE antibody

※8 According to Huby (2000), an allergen has at least two IgE-binding sites, each of which consists of a minimum of 15 amino acid residues. One molecule of allergen, therefore, must consist of 30 or more amino acid residues, which is about 3 kDa.

※9 The JECFA recommended a comparison of amino acid sequence on contiguous eight amino acid sequences in the Technical reports series 995.

※10 Comparison of amino acid sequence on contiguous amino acid sequences reveals the structural homology not only with B-cell epitope related to binding of IgE antibody but also with T-cell epitope related to sensitizing.

※11 Bioinformatics methods for comparison of the primary structure with that of the known allergens are to be the appropriate methods at the time based on the advances in science and technology.

of high titer against major allergens (eggs, milk, soy beans, rice, wheat, soba, cod, shrimp, crab, and peanuts) shall be selected.

- i) If the source organism is allergenic, a serum with specific IgE antibody of high titer against the organism shall be selected.
- ii) If the enzyme has a structural homology with known allergens, a serum with specific IgE antibody of high titer against the organism containing the allergen shall be selected.
- c) When potential concern of allergenicity cannot be excluded even based on the examination a) and b), potential allergenicity shall be comprehensively evaluated taking into consideration the data from clinical studies such as skin tests and oral tolerance tests.

4. Examination of the enzyme for the degradability in the gastro-intestinal tract and the allergenicity

It shall be clarified whether or not the following treatments 1) to 3) of the enzyme affect the characteristics of the enzyme, such as molecular weight, enzyme activity, and immunoreactivity.

This examination shall employ the methods such as SDS polyacrylamide gel electrophoresis, western blotting, ELISA method, or comparable methods.

- 1) Acid treatment and enzymatic (pepsin) treatment in simulated gastric juice
- 2) Alkaline treatment and enzymatic (pancreatin) treatment in simulated intestinal juice
- 3) Heat treatment (under the same or similar heating condition used in the treatment for human oral intake)

Article 3: Estimation of the Daily Intake

The daily intake of the enzyme added in the food shall be estimated in principle multiplying the daily intake of the subject food product^{*12} by the concentration of the enzyme.

Daily intake of a food shall be estimated appropriately based on the daily intake of each food group reported in the National Health and Nutrition Survey or other data. As for the used amount of the enzyme, assuming that the whole amount transfers into the end product and is consumed, the daily intake of the enzyme shall be estimated based on the maximal amount added under an ordinary usage. However, this calculation may result in an over-estimation if the enzyme is denatured/inactivated or degraded/removed. The body weight used for the estimation shall be the average body weight described in the latest decision of FSCJ.

*12 The food product in which the enzyme is to be used.

Appendix 1 : Abbreviations

Abbreviations	Explanations
CAS	Chemical Abstracts Service
EFSA	European Food Safety Authority
ELISA	Enzyme-Linked Immuno-Sorbent Assay
FSANZ	Food Standards Australia New Zealand
GLP	Good Laboratory Practice
JECFA	Joint FAO/WHO Expert Committee on Food Additives
OECD	Organisation for Economic Co-operation and Development
TOS	Total Organic Solids

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