Guidelines for Assessment of the Effect of Foods on Health for Food Additives

May 2010
Food Safety Commission

This document is an English version of the Guidelines for Assessment of the Effect of Foods on Health for Food Additives published by the Food Safety Commission, Cabinet Office of Japan in May 2010. Although the contents were reviewed by both Dr. Shoji Fukushima (Director, Japan Bioassay Research Center) and Dr. Dai Nakae (Head, Department of Pharmaceutical Sciences, Tokyo Metropolitan Institute of Public Health) for consistency with the original language, if there may be any concerns on the interpretation of any part of this document, please note that the original Japanese version should always be referred.
（http://www.fsc.go.jp/senmon/tenkabutu/tenkabutu-hyouka-shishin.pdf）

International Life Sciences Institute, Japan (ILSI Japan)

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I. GENERAL RULES

1. Background Leading to the Development of the Guideline

Food Safety Commission is required to prepare guidelines for assessment of the effect of food on health as stipulated in Article 21, paragraph (1) of the Food Safety Basic Act (approved by the Cabinet on Jan. 16, 2004), and has already developed the following: "Safety Assessment Standard for Genetically Modified Food (Seed Plants) (Jan. 29, 2004)", "Viewpoints on Assessment of the Effect of Food on Health for Official Specifications of General Fertilizer (Mar. 18, 2004)," "Safety Assessment Criteria for Food Additives Manufactured by Using Genetically Modified Microorganisms (Mar. 25, 2004)," "Viewpoints on Safety Assessment of Genetically Modified Feed and Feed Additives (May 6, 2004)," " Guidelines for Assessment of the Effect of Food on Health for Selected Drug-Resistant Microorganisms by Using Antimicrobial Substance for Livestock (Sep. 30, 2004)," and "Safety Assessment Standard for Genetically Modified Food (Microorganisms) (June 26, 2008)."

Guidelines for Assessment of the Effect of Food on Health is considered necessary in order to ensure scientifically valid conclustions and impartiality during the assessment of the effects of food on health, and to specify the necessary data required from applicants thereby ensuring the transparency of the process, both domestically and internationally.

Food Safety Commission has established the Guidelines for Assessment of the Effect of Food on Health for Food Additives is based on the results of past assessments of the effects of food on health related to food additives and on domestic and international opinions and standards related to safety assessment, and therefore future assessments will be conducted in accordance with these guidelines.

Global trends related to assessment criteria as well as new scientific findings both from Japan and abroad will be used will be used to reassess the provisions of these guidelines, and any necessary revisions will be made.

2. Definitions

1. Food Additive
A substance used for food by way of addition, mixture, infiltration and other methods, in the course of food manufacture, or with the purpose of food processing or preservation, as prescribed in Food Sanitation Act (Act No. 233 of 1947) Article 4, paragraph (2).

2. ADI: Acceptable Daily Intake
The daily intake level of a substance that is presumed to have no adverse effect on health as judged by present scientific knowledge, even if the substance is continuously taken every day

over the lifetime of a person.

3. UL: Tolerable Upper Intake Level
The dose of a substance which provides the highest habitual intake that is presumed to pose no risk of health disturbance.

4. NOAEL: No Observed Adverse Effect Level
The highest dose of a substance without observed adverse effect when a toxicity study is performed using several dose levels.

5. LOAEL: Lowest Observed Adverse Effect Level
The lowest dose of a substance with observed adverse effect when a toxicity study is performed using several dose levels.

6. BMD: Benchmark Dose
The dose of a substance that is expected to result in a prespecified level of toxicity, calculated by applying mathematical models for the correlation between toxicity incidence and dose.

7. VSD: Virtually Safe Dose
The dose of a substance that is presumed to cause cancer at a "risk level" (a low probability such as 10^{-5} or 10^{-6}) when the maximum tolerated residue level of a substance is continuously taken every day over the lifetime of a person; a parameter used in an evaluation method starting from the viewpoint that there is no threshold value for the carcinogenicity of genotoxic carcinogens.

8. Toxicity Index (Endpoint)
An observable or measurable biological event or a chemical concentration used as an index to measure effects of exposure to a target substance for evaluation.

9. Safety Factor
A factor used to set the ADI of a substance from NOAEL in order to ensure sufficient safety.

10. MOA: Mode of Action
The mechanism of action a substance exerts on an organism.

11. Evaluation Based on WOE (Weight of Evidence)
An evaluation conducted by paying attention to the importance of information used as evidence.

12. GLP: Good Laboratory Practice

A set of standards for facilities, equipment, organization, personnel, operation procedures and others that a laboratory must furnish to ensure the reliability of the results of various safety studies on chemical substances.

13. Epidemiology
A field of science that measures the incidence and distribution of various problems related to the health of human populations, and factors affecting such problems (e.g. food, smoking and drinking), for developing effective measures against health-related problems.

14. JECFA: Joint FAO/WHO Expert Committee on Food Additives
An expert committee organized jointly by FAO and WHO; that conducts risk assessments for food additives, contaminants, veterinary medicines etc., and provides scientific advise to FAO, WHO, member countries and the Codex Alimentarius Commission.

15. The 1996 Guideline of the Ministry of Health and Welfare
Guideline for Designation of Food Additives and Revision of Standards for Use of Food Additives (EHB/FCD Notification No.29 of March 22, 1996).

16. Universally-used Food Additives
Food additives 1) for which the safety has been confirmed to a certain extent on the basis of completed JECFA international safety assessments, and 2) of which are widely used and accepted in the US and EU countries and globally considered to be highly necessary, according to a meeting of the Food Sanitation Subcommittee of Pharmaceutical Affairs and Food Sanitation Council held in July, 2002. The Ministry of Health, Labour and Welfare announced a policy to start considering designation of these food additives without waiting for applications from companies.

3. Aim

The purpose of this guideline is to provide guidance for evaluation and the scope of documents necessary for the assessment of the effect of food on health, in order to determine whether food additives pose any risk to human health in accordance with Article 10 of the Food Sanitation Act, and to establish the specifications and standards for food additives in accordance with Article 11, paragraph (1) of the same Act.

An assessment should be conducted in accordance with these guidelines,when a food additive is to be deleted from the existing food additives list in accordance with Article 2-2, paragraph (1) of Supplementary Provisions of The Act for Partial Revision of the Food Sanitation Act and the Nutrition Improvement Act (Act No. 101 of 1995).

4. Viewpoints on Assessment of the Effect of Food on Health for Food Additives

1. Food Safety Commission will organize its standpoint on safety standards and then apply these to the assessment of the effects of food on health for food additives in the future. At present, the handling of the safety standards is entrusted to an evaluation by the expert committee.

2. The assessments of the effect of food on health for so-called universally-used food additives (excluding universally-used flavoring agents), for which safety assessments by JECFA have been completed, and for which use has been accepted for a long period of time in the US and European countries, are conducted principally based on the evaluation reports made by JECFA and in US and European countries (assessment based on evaluation reports), supplemented by a review of the latest scientific knowledge.

3. Regarding food additives that are genotoxic carcinogens[1], their assessments of the effect of food on health are principally conducted according to the viewpoint that a threshold value does not exist at present, because the international discussion for its existence has not as yet reached a consensus. It is necessary to judge carefully whether a particular substance is a genotoxicic carcinogen or not by considering both the MOA and WOE of the substacnce.

4. At present, a food additive evaluated as a genotoxicic carcinogen in principle should not be approved in accordance with the preceding paragraph. However, when impurities (including natural substances) or by-products, which are unavoidably present during production are genotoxicic carcinogens, the amount should be reduced as low as technically possible, and a comprehensive assessment conducted based on criteria such as VSD.

5. For substances intended to be used as an alternatives to regular food components, as foods with nutrient function claims, or for the purpose of nutritional enrichment, their assessment is to be performed from a nutritional viewpoint, considering their quality as nutrient components and their intake from other foods, with reference to "Dietary Reference Intakes".

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[1]Genotoxicic carcinogen is a substance where the substance itself or its metabolite is considered to cause genetic mutations or clastogenetic properties due to direct action on DNA, and actions related to the genotoxicity are considered to be a part of its carcinogenic mechanism. It is necessary that the the genotoxicity be confirmed *in vivo* (if possible, as the target organ for the carcinogenesis).

6. A special examination for pregnant women and fetuses, infants, children and the elderly is performed if necessary, for instance in cases where the data suggest a potential risk.

7. According to the necessity, it is advisable to examine the usefulness of *in vitro* studies conducted in other areas such as the development of medicines and apply these to food additives. One example is a case in which findings in animals was extrapolated to humans where a concern about adverse effects due to metabolites found in animal studies and was examined by *in vitro* studies using human drug-metabolizing enzymes.

8. When a food additive subject to the assessment is considered to interact with medicines, individuals potentially affected are basically thought to be under medical monitoring. Studies into the interactions with medicines will be conducted as needed, when potential risks are known.

9. The necessity of evaluating decomposition products, concomitant impurities and human metabolitess of food additives will be considered. The stability of food additives and their stability in food will be confirmed as well. When they are not stable, the decomposition products and their levels will also be examined.

10. Regarding adverse effects from the combined intake of multiple food additives, it is considered possible to ensure the practical levels of safety by conducting sufficient evaluations of individual food additives, based on the Report of the Food Safety Commission on the 2006 Comprehensive Food Security Survey "Information Collection about Combined Effects of Food Additives". . However, the evaluations will be performed as needed, when potential risks are known.

11. Care should be taken in the handling of the results of studies using genetically modified animals, because such results have rarely been utilized in the evaluation by JECFA and other organizations, even though there have been a few cases in which the Food Safe Commission used such results for risk assessments.

12. JECFA considers food additives manufactured using new technologies, such as nanomaterials, may have different toxicological properties, and that in general past standards or ADI may not be able to be applied to them. When an evaluation for such substances is nessecary, an appropriate assessment will be conducted.

5. Viewpoints on Documents Required for Assessment

1. Scope of documents necessary for the evaluation and points to consider are shown in

Chapter II Detailed Exposition, and Attached Tables 1 and 2, while they are also shown as follows. In principle, specific examination methods will be in compliance with internationally-recognized testing guidelines including those of the Organization for Economic Cooperation and Development (OECD).

(1) Specific tests can be omitted, when it is scientifically demonstrated that the food additive is a resident food component or becomes so via degradation in the food or in the digestive tract. Whether it is scientifically demonstrated or not will be judged by considering Table 2 of the 1996 Guideline of the Ministry of Health and Welfare.

(2) Evaluation will be performed considering its long eating experience in humans, when the food additive is a universally-used additive (refer to Chapter I, Section 4-2), and considering the characteristics of the substance, when the additive is a universally-used flavoring agent, enzyme or nutrient component (refer to Chapter II, Sections 5, 6, and 7).

(3) A part of tests can be omitted, when the food additive is different only at its base moiety from, or an isomer of the already approved food additive, or when there are other scientifically reasonable reasons, but the exact reason should be presented.

2. Points to consider during the revision of the standards for use or the component specifications are as follows.

(1) The following points should be considered during the revision of the standards for use.

i) When the assessment of the effect of food on health by the Food Safety Commission has been finished for the food additive, the documents regarding the estimation of the daily intake based on the addition of the requested food for use, and the change in used quantity should be submitted. When there is a new toxicological finding, the documents regarding the finding should also be submitted.

ii) When an assessment of the effect of food on health by the Food Safety Commission has not yet been performed for the food additive, documents necessary for the assessment for designation of food additive should in principle be submitted.

(2) In the case of a revision of the component specifications, it is necessary to demonstrate that there will be no problems concerning validity and safety of the component specifications being revised.

3. Documents necessary for the assessment should be submitted by and are the responsibility of the applicant, and the reliability of the contents of the documents should be ensured by the

applicant as well. In principle, the applicant should submit results of studies conducted at testing facilities recognized to be properly administered (facilities qualified with GLP) and using test methods for which reliability is guaranteed, scientifically reliable documents such as evaluation reports issued by international organizations *etc*. However, documents indicating concerns about the safety of the food additive should be submitted regardless of their reliability, because such documents may be required for the assessment.

4. It is recommended that necropsy and histopathological evaluation be conducted by individuals with sufficient experience.

5. Raw data and animal study specimens should be stored for the period specified by GLP or until the completion of the evaluation, and they should be submitted as necessary.

6. In principle, the assessment uses documents submitted by the applicant, and additional documents may be requested from the applicant, when documents necessary for the assessment are judged to be insufficient.

6. Interpretation of Toxicokinetics and Toxicity Studies

A toxicokinetics study is conducted to evaluate *in vivo* absorption, distribution, metabolism, and excretion, when humans intake the food additive. It is, therefore, insufficient for the results of the animal study to only be summarized, but the toxicokinetics and its relationship with the induction of adverse effects in humans should also be discussed.

When interpreting study data, it is necessary to scientifically discuss whether the observed toxicity, *in vivo* persistence *etc*. is an accidental result other, such as nutritional status, or is instead truly a property of the food additive. For the determination of endpoints, rationally scientific interpretation should be made on the statistical significance and the dose dependency of each test of related findings such as general signs, body weight, food intake, blood examinations, blood biochemistry, urianalysis and pathological examinations, by considering differences of animal species and doses among toxicokinetics and toxicity studies. Furthemore, the mode of action of the toxicity should be described as much as possible.

7. Risk Judgment

1. Viewpoints on Establishing an ADI

Basic viewpoints on the establishment of an ADI are as follows.

(1) When an ADI is established based on plural NOAELs through the general evaluation of toxicological study results, the lowest NOAEL is principally chosen for the basis after

comparing data within each animal species or toxicity study.

(2) In principle, the results of toxicity studies are evaluated considering the gender differences, and NOAELs are set separately for males and females.

(3) A safety factor is basically 100 (species difference 10 and individual difference 10), considering species and individual differences. However, the safety factor 100 can be changed if necessary, and a different safety factor set by considering toxicity and study data as follows.

i) When human study data are used, there is no need to consider species differences. Considering individual differences, a safety factor in the range of 1 to 10 is employed depending on the numbers of the study population *etc*.

ii) When sufficient information is not available, or in the case where the food additive subject to the assessment shows serious toxicity[2], anadditional safety factor in the range of 1 to 10 for each factor will be applied.

iii) When an ADI is set based on LOAEL, an additional safety factor in the range of 1 to 10 is employed. Alternatively, a benchmark dose may be used.

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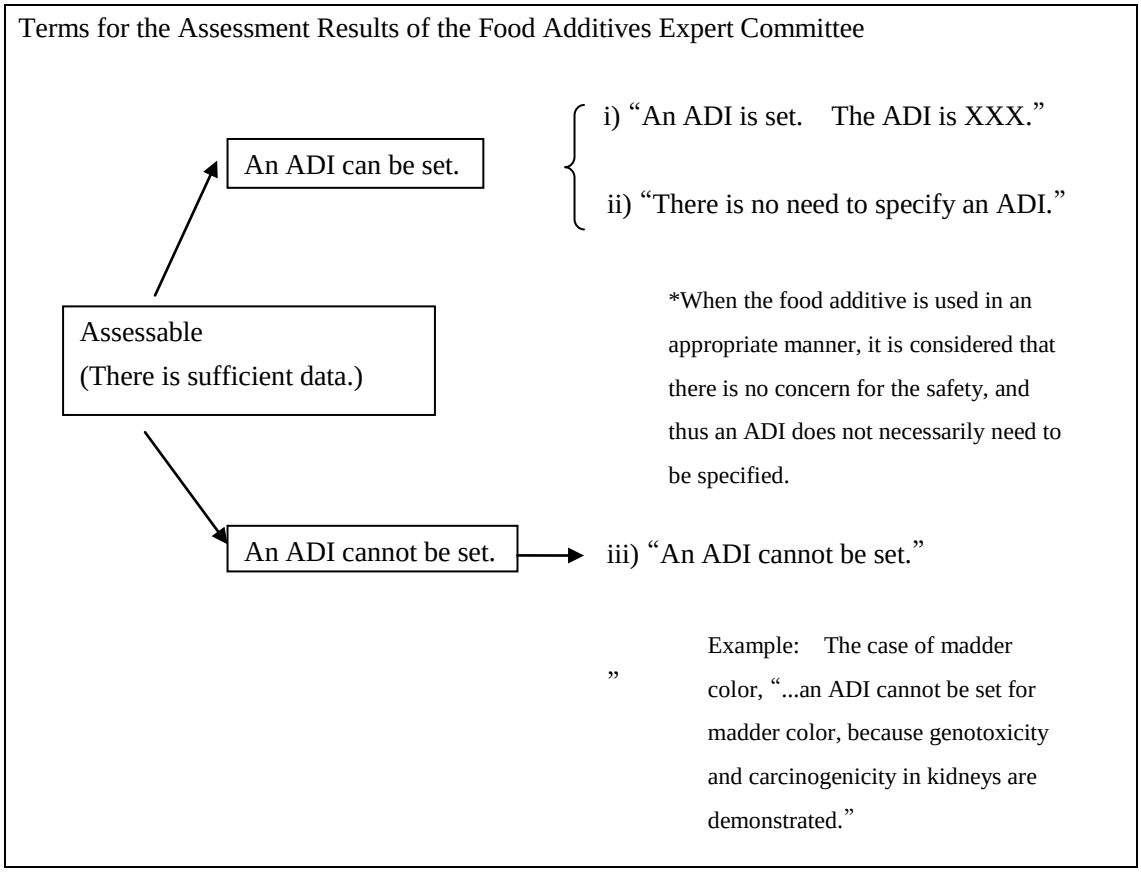
[2]"Principles for the Safety Assessment of Food Additives" (IPCS, EHC 70) presents the following exmaples.

a) Irreversible effects observed in prenatal development toxicity studies

b) Findings of carcinogenicity

(4) Terms related to the assessment result should be employed as shown below.



2. Determination of NOAEL

On the determination of a NOAEL in a particular study, it is required to examine whether appropriate doses have been set. Specifically, in a toxicological study, the highest dose should be the dose where toxic effects are observed, the lowest dose should be the dose where no toxic effects are observed, and each dose level should be established to observe a dose-response relationship. However, when administered dietarily, consideration should be made to prevent malnutrition, and thus usually it is not necessary to conduct a study with doses higher than 5% (W/W). In the case of the gavage administration, when no toxicological effects are observed at the highest level technically possible or at 1,000 mg/kg body weight, it is not necessary in general to conduct a study with higher doses.

When two or more studies are conducted using different animal species, a NOAEL is derived in each study. To calculate an ADI, the NOAEL derived from the animal study that showed toxic effect at the lowest dose should be used. However, when a particular study is clearly more appropriate than

other studies in terms of it design and results, and when studies with different periods are conducted, a special weight should be put on a longer and more appropriate study to identify a NOAEL for calculating an ADI. When metabolic and pharmacodynamic data are available, it is possible to derive a NOAEL to calculate an ADI based on a study using animal species for which toxicological responses are most similar to those in humans.

3. Group ADI

An ADI is set for a group of substances used as food additives to manage their cumulative intakes, when the substances have a similar structure-activity relationship, or when even if they do not have such a relationship, they can display toxicity in a similar range and can produce additive physiological and toxicological effects. When setting a group ADI, the lowest NOAEL among NOAELs of substances in the group should be used. Furthermore, relative data quality and periods of the toxicity study should be considered to set a group ADI. When only one NOAEL of a particular substance in a group is quite different from other NOAELs, the substance should be excluded from the group.

8. Reassessment

Even for an approved food additive, the possibility of the appearance of adverse effects should be continuously monitored, When an adverse effect is discovered by advances in toxicology, for example, the food additive should be reassessed.

When new and important data related to the safety of an approved food additive becomes known, the food additive should be promptly reassessed.

II. DETAILED EXPOSITION

Documents necessary for the assessment are shown in Attached Tables 1 and 2. Details are described as follows.

1. Summary of Food Additives Subject to Assessment

1. Name and Use

2. Origin or Chronology of the Development

3. Foreign Conditions of Use

4. Evaluation by International Organizations *etc*.

5. Physicochemical Characteristics

Chemical name (Japanese name, English name, CAS number), molecular formula, molecular weight, structural formula, methods of manufacture, properties, stability (including stability in food), component specifications draft *etc*.

6. Draft of Standards for Use

(1) When it is judged to be necessary to establish standards for use to comprehensively examine the safety and effectiveness of a food additive, and to limit the food for use, consumed quantity *etc*., it is necessary to demonstrate the basis for establishment. Upon establishment, considerations should be made on issues such as the comparison between an estimated daily intake (refer to Chapter II, Section 4) and an ADI calculated from toxicity studies.

(2) When it is judged that there is no need to establish standards for use, the basis should be specified.

7. Others (Information Useful for the Assessment of the Effect of Food on Health)

2. Studies on Safety

1. Toxicokinetics Study

The "Toxicokinetics Study" in the 1996 Guideline of the Ministry of Health and Welfare, and the issues below should be followed.

(1) As a test substance, a food additive or its isotope-labeled substance is used. When the latter is used, information for labeled nuclide, labeled position *etc*. are specified.

(2) It is advisable that studies be conducted in a total of 2 or more species, 1 or more rodent species (usually rats) and 1 or more non-rodent species (usually dogs).

(3) Route of administration is principally *per os*. Absorption, distribution, metabolism and excretion are evaluated by conducting single dose and repeated dose administrations. In order to calculate an exact absorption rate, additional studies are preformed if necessary, for example studies featuring intravenous administration.

(4) To evaluate each step of absorption, distribution, metabolism and excretion, data is required to represent the blood concentration of the active ingredient, quantity of excretion

to urine and feces, time-dependent change of concentration in each organ, *in vivo* metabolites and factors affecting each step.

(5) Potential target organs in toxicity studies are estimated from the results of absorption, distribution, metabolism and excretion (e.g. the maximum plasma concentration, time-dependent change of concentration in each organ, elimination half-life *etc*.). On this occasion, it is necessary to consider species difference and species specificity, and to discuss the extrapolability to humans.

(6) When the test substance is in a racemic form, it is advisable to investigate toxicokinetics of each optical isomer, if necessary with regard to the toxicity.

(7) In principle, it is evaluated whether characteristic metabolites in humans are produced or not, and if yes, toxicity studies of the metabolites are conducted as necessary.

2. Toxicity Studies

(1) Subacute toxicity test and chronic toxicity studies

i) The test is conducted using 1 rodent species (usually rats) and 1 non-rodent species (usually dogs). In principle, the same numbers of male and female animals are used.

ii) Administration period is 28 and 90 days for the subacute toxicity study, and 12 months or more for the chronic toxicity study. However, a 28-day study can be omitted, when a 90-day study is performed.

iii) The test substance is administered orally 7 days a week. In principle, the test substance is given dietarily or by admixing into the drinking water, but if difficult, a gavage administration may be adopted.

iv) There should be at least 3 groups to which the test substance has been administered at different dose levels, other than the control group. The basis for choice of dose levels should be defined, and a common ratio is set to obtain an appropriate NOAEL.

v) When the test substance is administered dietarily, consideration should be addressed to avoid malnutrition, and usually it is not necessary to conduct a study with concentrations of the test substance in the diet more than 5% (W/W). In the case of a gavage administration, it is not necessary to conduct a study with higher doses in general, when no toxic effect is observed at the highest dose technically possible or at 1,000

mg/kg body weight.

vi) When an increase of frequency or severity of spontaneous lesions is also observed in the control group is principally considered to be an effect caused by the administration of the test substance, when the biological significance, such as the dose-effect relationship, is present, even if the increased level is within the range of the background data.

vii) When neurotoxicity or immunotoxicity[3] is suspected, additional studies should be conducted according to the OECD test guidelines, the ICH (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use) guidelines *etc*.

viii) It is required to consider carefully how to extrapolate the findings recognized in the toxicity studies to humans, by examining endpoints separately as functional changes, non-neoplastic morphological changes, neoplastic changes, reproductive functional changes *etc*.

ix) When a combined chronic toxicity and carcinogenicity study is performed using 1 rodent species, a chronic toxicity study using 1 rodent species can be omitted.

x) An additional in-utero exposure phase should be considered if necessary.

(2) Carcinogenicity study

i) The test is conducted using 2 or more rodent species (usually rats, mice, or hamsters). In principle, the same numbers of male and female animals are used.

ii) Administration period is 24 to 30 months for rats, and 18 to 24 months for mice. The test substance is administered orally 7 days a week. In principle, the test substance is given dietarily or by admixing into the drinking water, but if this is not practical, a gavage administration may be adopted.

iii) At least 3 groups administered the test substance with different dose levels are set, other than a control group. The basis to choose dose levels should be defined, and the common ratio is set to obtain an appropriate NOAEL.

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[3]This immunotoxicity means the toxicity resulting from the unintentional and non-antigen-specific suppression or enhancement of the immune function by the test substance taken into the body.

iv) When the test substance is administered dietarily, consideration should be addressed to avoid malnutrition, and usually it is not necessary to conduct a study with concentrations of test substance in the diet more than 5% (W/W). In the case of gavage administration, it is not necessary to conduct a study with higher doses in general, when no toxic effect are observed at the highest dose technically possible or at 1,000 mg/kg body weight.

v) When both the carcinogenicity and the genotoxicity are positive, the test substance is judged as a genotoxic carcinogen, and thus an ADI cannot be established in principle. However, if the genotoxicity is negative, indicating that the test substance is a non-genotoxic carcinogen, an ADI can be set. When impurities or by-products suspected to be genotoxic are unavoidably produced or are residual in the food additive subject to the assessment, it may be possible to set an ADI of the food additive after necessary examination (refer to Chapter I, Section 4-3, 4).

vi) When incidences of proliferating lesions are relatively low, the carcinogenicity may be evaluated by analyzing the statistical significance of the combined incidence of benign and malignant tumoral lesions, or that of preneoplastic, benign tumoral and malignant tumoral lesions. Especially with respect to the increase of endocrine tumors which commonly occurs in rodents, it is desirable to evaluate the carcinogenicity by including preneoplastic lesions in the analysis.

vii) It is desirable to evaluate the carcinogenicity including its underlying mechanisms, even when the increase of a tumor is noted at sites that are not common, or when the increase of rare tumor is noted.

viii) The assessment is conducted taking factors modifying carcinogenesis (*e.g.*, the suppression of the body weight gain or the decrease of survival rate) into consideration.

ix) Consideration should be addressed for toxic findings specific to particular animal species (*e.g.*, the hypertrophy, hyperplasia and tumor of the thyroid follicular epithelium specific to rodents, and the disorder and tumor of the kidney specific to male rats).

x) When a combined chronic toxicity and carcinogenicity study is performed using 1 rodent species, a carcinogenicity study using 1 rodent species can be omitted.

xi) An addition of an in-utero exposure phase should be included if necessary.

(3) Combined 1-year repeated dose toxicity and carcinogenicity study

The points of consideration in (1) and (2) should be followed.

(4) Reproductive toxicity study

The "Reproduction Study" in the 1996 Guideline of the Ministry of Health and Welfare, and the issues below should be followed.

i) The test is conducted using 1 or more rodent species (usually rats). In principle, the same numbers of male and female animals are used.

ii) The test substance is administered orally 7 days a week. In principle, the test substance is given dietarily or by admixture into drinking water, but if this is prohibitively difficult, gavage administration may be used.

iii) Administration of the test substance to at least 3 groups at different dose levels, other than the control group. The basis for choose of dose levels should be determined, and the common ratio set to obtain an appropriate NOAEL.

iv) When the test substance is administered dietarily, care should be taken to avoid malnutrition, and usually it is not necessary to conduct a study with concentrations of the test substance in the diet more than 5% (W/W). In the case of gavage administration, it is not necessary to conduct a study with higher doses in general, when no toxic effect is observed at the highest dose technically possible or at 1,000 mg/kg body weight.

v) When neurotoxicity or immunotoxicity is suspected, it is considered necessary to conduct additional studies according to the OECD test guidelines, the ICH guidelines and *etc*.

(5) Prenatal development toxicity study

The "Teratogenicity Study" in the 1996 Guideline of the Ministry of Health and Welfare, and the issues below should be followed. The administration period is at least from the implantation day to the day before the estimateed parturition day, and the test substance should administered daily to pregnant animals.

i) The study is conducted using a total of 2 or more speciesl, 1 or more rodent species (usually rats) and a non-rodent species (usually rabbits).

ii) The test substance is administered by gavage.

iii) Administration of the substance to at least 3 groups using different dose levels, in addition to the control group. The basis to choose dose levels should be defined, and a common ratio set to obtain an appropriate NOAEL.

(6) Genotoxicity study

The "Mutagenicity Study" in the1996 Guideline of the Ministry of Health and Welfare should be followed, but the evaluation should be performed based on the test results relating to the overall genotoxicity, and not be limited to those relating to "mutagenicity" in a narrow sense. Among the tests which form the standard combination (the "reverse mutation test using microorganisms", the "chromosomal aberration test using cultured mammalian cells" and the "micronucleus test using rodents"), the "chromosomal aberration test using cultured mammalian cells" can be substituted by the mouse lymphoma TK assay (MLA) or the *in vitro* micronucleus test. Examples of additional tests to complement the results the standard combination include the single cell gel electrophoresis test (comet test), the *in vivo* transgenic animal mutation assay, in addition to those exemplified in the 1996 Guideline of the Ministry of Health and Welfare.

However, when any of tests of the standard combination cannot be conducted because of technical limitations, the reason should be explained based on scientific rationale. The evaluation should be conducted using the data of the substituting test of which validity is confirmed by international validation.

A procedure to judge test results are as follows.

i) When the result of the "reverse mutation test using microorganism" is positive, the comprehensive judgment is made by sufficiently considering the results of the *in vivo* tests which are parameters for genetic mutation or the DNA damage (such as the comet test and the *in vivo* transgenic animal mutation assay).

ii) When the result of the "chromosomal aberration test using cultured mammalian cells" is positive, and this is confirmed by the "micronucleus test using rodents," the genotoxicity can be judged as positive.

iii) Even though the result of the "chromosomal aberration test using cultured mammalian cells" is positive, if the result of the "micronucleus test using rodents" conducted using an appropriately high dose (It is desirable that the target organs are proved to be exposed.) is negative, the genotoxicity can be judged as negative.

(7) Allergenicity study

The "Antigenicity Study" in the 1996 Guideline of the Ministry of Health and Welfare is referred. Methods to predict the allergenic potential of orally administered chemical substances, especially those with the potential to induce the immediate hypersensitivity, are not yet well established. The study should be conducted using the sensitization and elicitation methods that are approved to be appropriate by experts, taking into account all known information and typical use of the food additive.

For the time being, it is necessary at least to conduct the allergenicity study targeting delayed allergy. Usable studies include the skin sensitization test using guinea pigs (*e.g.*, the maximization test (GPMT) of the OECD Test Guideline 406) and the reactive lymph node test using mice (*e.g.*, the OECD Test Guideline 429 (the local lymph node assay (LLMA)).

The assessment of the allergenicity of food additives for which the constituent is protein is conducted by following the "Safety Assessment Standard for Genetically Modified Food (Microorganisms) (decided by the Food safety Commission, June 26, 2008)".

(8) General pharmacological study

The "General Pharmacological Study" in the 1996 Guideline of the Ministry of Health and Welfare Guideline should be followed.

(9) Other studies

When neurotoxicity is suspected as a result of the subacute toxicity study or other studies, additional studies should be conducted as needed according to the OECD test guidelines and *etc*.

When immunotoxicity is suspected in the subacute toxicity study or other studies, appropriate immune function studies should additionally be conducted as needed according to the ICH test guidelines and *etc*. An immune function study may also be requested, when potential immunotoxicity in humans is suspected from known information.

3. Findings in Humans

Data from clinical and epidemiological studies is applied when available. When allergenicity is suspected, human data is preferred, because it is often difficult to extrapolate the results of animal study to humans.

4. Estimate of Daily Intake

1. Daily intake of the food additive in Japan is estimated. In this estimation, the intake should not be underestimated. In principle, the estimate is calculated by multiplying the daily intake of the food for use by the content of the food additive. The daily intake of the food should be estimated from the Intake of Each Food Group demonstrated in the National Health and Nutrition Examination Survey, or other appropriate documents. The estimation can be conducted using data obtained by reliable techniques such as the market basket survey and the survey based on Production Statistics. The estimated daily intake is calculated assuming that the body weight of an individual is 50 kg.

2. The estimated daily intake is compared with the ADI derived from toxicity studies. If necessary, the safety of the case in which the same sort of food additives are taken together should be examined, by comparing the cumulative estimated daily intake with the group ADI.

3. Based on actual food intake in Japan, the influences of the excessive intake of nutrient components and of electrolyte balance should also be included.

5. Evaluation Method for Universally-used Flavoring Agents

The evaluation is performed based on the "Method of Safety Assessment for Universally-used Flavoring Agent (Final Report and Re-revised Edition) (November 4, 2003)". In this assessment, the genotoxicity is assessed *in vitro* using microorganisms and mammalian cells, and *in vivo* tests are performed, when the *in vivo* genotoxicity is suspected. However, if the results of the *in vivo* micronucleus test have already been obtained, it is not necessary to conduct the *in vitro* chromosomal aberration test.

In order to estimate the intake, JECFA shows its stance that in addition to the traditional PCTT (Per Capita intake Times Ten) method, a technique to estimate the total intake based on the estimation of the food for use and the content of the food additive in each food group (SPET or Single Portion Exposure Technique method) should be used, and these results taken into account in all further testing.. In Japan, however, it is difficult to estimate the content of the food additives according to the new designation. The assessment is therefore being conducted using the traditional PCTT

method for the time being, and the application of SPET is treated as a subject for future investigation.

6. Evaluation Method for Enzymes

The safety assessment for enzymes is principally conducted using data shown in Appendix 1. However, when an enzyme is derived from microorganisms, and when the safety of the strain producing the enzyme is not clear, it is necessary to conduct adequate tests and evaluate the safety of the source microorganism. Virulent or toxin-producing strains should not in principle be used for production of the enzyme.

When it is scientifically demonstrated that the enzyme is degraded in the gastrointestinal tract into resident food components (judged after considering issues listed in Table 2 of the 1996 Guideline of the Ministry of Health and Welfare), documents concerning toxicity and listed in Appendix 1 can be omitted, but documents concerning toxicity and listed in Appendix 2 should be submitted.

7. Evaluation Method for Nutrient Components

Documents necessary for the safety assessment of a nutrient component for which intake is biologically essential, or which has been proven to exert effects beneficial to health by the intake at the specified level, are shown in Appendix 1. The assessment is conducted referring to "A Model for Establishing Upper Levels of Intake for Nutrients and Related Substances" (Report of a Joint FAO/WHO Technical Workshop on Nutrient Risk Assessment, WHO Headquarters, Geneva, Switzerland, 2-6 May 2005).

The assessment for a nutrient component is performed with consideration to the following issues.

1. The assessment should be conducted comprehensively based on human findings obtained by clinical studies, epidemiological studies and case reports. On this occasion, knowledge obtained from meta-analysis is used while considering the variability of background factors and the research quality of these reports.

2. The range of the required level and the intake of the nutrient component in humans is often close to LOAEL or NOAEL reported in humans. It is thus necessary to apply a different uncertainty factor[5] for each nutrient component, considering the homeostatic function specific to the nutrient component.

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[5]A safety factor used to set a tolerable upper level of the intake of a nutrient in the Dietary Reference Intakes.

3. When the background habitual intake from food must be considered, for instance in cases where there is a concern that excessive intake may cause a serious impact on human health, not only the mean value but also the distribution of the habitual intake should be examined as necessary.

4. For a nutrient component for which the tolerable upper level of the intake is shown in the "Dietary Reference Intakes for Japanese" developed by the Ministry of Health, Labour and Welfare, such a level and its background data are to be considered.

Appendix 1. List of Documents Required for the Assessment of Food Additives (Excluding the Cases of Universally-used Flavoring Agents)

Document Category		Designation	Revision of Standards
Summary of Food Additive Subject to Assessment			
	1. Name and Use	○	○
	2. Origin or Circumstances of Discovery	○	Δ
	3. Usage in Foreign Countries	○	○
	4. Evaluation by International Organizations	○	Δ
	5. Physicochemical Properties	○	Δ
	6. Draft of Standards for Use	○	○
	7. Others	Δ	Δ
Findings Relating to Safety			
	1. Toxicokinetics Study	○	Δ
	2. Toxicity Study		
	(1) Subacute toxicity and chronic toxicity study	○	Δ
	(2) Carcinogenicity study	○	Δ
	(3) Combined 1-year repeated dose toxicity and carcinogenicity study	○	Δ
	(4) Reproductive toxicity study	○	Δ
	(5) Prenatal development toxicity study	○	Δ
	(6) Genotoxicity study	○	Δ
	(7) Allergenicity study	○	Δ
	(8) General pharmacological study	○	Δ
	(9) Other stuidies	Δ	Δ
	3. Information in humans	○	Δ
	4. Estimated Daily Intake and *etc*.	○	○

(Note 1) At the revision of the standards for the use of the food additive for which the assessment for the effect of food on health by the Food Safety Commission was performed, documents for the "Revision of Standards" should be submitted. On the other hand, documents for the "designation" should principally be submitted for food additives for which such an assessment has not been performed.

(Note 2) Documents with open circles should be attached, while documents with triangles should be attached when necessary, such as the case that a new finding is obtained.

(Note 3) When the combined chronic toxicity and carcinogenicity study is performed in 1 rodent species, the chronic toxicity study and carcinogenicity study in 1 rodent species may be omitted.

Appendix 2. List of Documents Regarding Toxicity Required for the Assessment of Enzyme (When It Is Scientifically Demonstrated That The enzyme Is Degraded in the Gastrointestinal Tract into Resident Food Components, after Considering Issues Listed in Table 2 of the 1996 Guideline of the Ministry of Health and Welfare)

Document Category	Designation	Revision of Standards
(1) 90-day Repeated Dose Toxicity Study in Rodents	○	Δ
(2) Genotoxicity Study	○	Δ
(3) Allergenicity Test	○	Δ

(Note) In order to evaluate allergenicity, necessary tests are to be conducted according to the "Safety Assessment Criteria for Genetically Modified Food (Microorganisms) (June 26, 2008, the Food Safety Commission Decision)" for the time being.