

Provisional translation

The Food Safety Commission

Final decision on July 18, 2017

This English version of the Commission Decision is intended to be reference material to provide convenience for users. In the event of inconsistency between the Japanese original and this English translation, the former shall prevail. The FSCJ shall not be responsible for any consequence resulting from use of this English version.

Guidelines for the Risk Assessment of Food Additives
(Amended Guidelines for Assessment of the Effect of Food on
Human Health Regarding Food Additives)

May 2010

(Amended in July 2017)

Food Safety Commission of Japan (FSCJ)

Table of Contents

| | Page |
|--|-----------|
| <History of Evaluation>..... | 3 |
| <Food Safety Commission Members>..... | 4 |
| <Food Safety Commission Food Additives Expert Committee Members>... | 5 |
| Chapter I. General Provisions | 7 |
| Article 1. Background | 7 |
| Article 2. Definition | 8 |
| Article 3. Purpose | 9 |
| Article 4. Approach for the Risk Assessment of Food Additives | 10 |
| Article 5. Approach for the Documents Required | 11 |
| Article 6. Toxicokinetics and toxicity study | 13 |
| Article 7. Risk determination | 13 |
| 1. Approach for establishing ADI | 13 |
| 2. Determination of NOAEL..... | 14 |
| 3. Group ADI..... | 15 |
| Article 8. Re-evaluation | 15 |
| | |
| Chapter II. Detailed Expositions | 16 |
| Article 1. Outline of the Target Additives | 16 |
| Article 2. Information relevant to safety | 16 |
| 1. Toxicokinetics | 16 |
| 2. Toxicity study..... | 17 |
| (1) Subchronic toxicity study and chronic toxicity study | 17 |
| (2) Carcinogenicity study..... | 18 |
| (3) Combined one-year repeated dose toxicity/carcinogenicity study | 20 |
| (4) Reproductive toxicity study..... | 20 |
| (5) Prenatal developmental toxicity study..... | 20 |
| (6) Genotoxicity study..... | 21 |
| (7) Allergenicity study..... | 22 |
| (8) General pharmacology..... | 22 |
| (9) Other studies | 22 |
| Article 3. Findings in humans | 22 |
| Article 4. Estimation of daily intake | 23 |

Article 5. Assessment of Flavors..... 23
Article 6. Assessment of Enzymes 23
Article 7. Assessment of Food Additives for Fortification 23

Appendix 1. Documents required for assessments of food additives 25

<History of Evaluation>

Establishment of Guidelines

- November 11, 2008: 64th meeting of the Food Additives Expert Committee
December 15, 2008: 65th meeting of the Food Additives Expert Committee
January 13, 2009: 66th meeting of the Food Additives Expert Committee
February 2, 2009: 67th meeting of the Food Additives Expert Committee
March 23, 2009: 69th meeting of the Food Additives Expert Committee
April 20, 2009: 70th meeting of the Food Additives Expert Committee
May 18, 2009: 71st meeting of the Food Additives Expert Committee
September 7, 2009: 77th meeting of the Food Additives Expert Committee
October 22, 2009: 306th meeting of FSCJ (report by the Food Additives Expert Committee)
October 22 to November 20, 2009: Public comments
December 15, 2009: 81st meeting of the Food Additives Expert Committee
May 25, 2010: Report to the Chairperson of FSCJ by the Chairperson of the Food Additive Expert Committee
May 27, 2010: 333rd meeting of FSCJ (reporting of the discussion results from the Food Additive Expert Committee meeting)
Adopted and published as the Guideline for Assessment of the Effect of Food on Human Health Regarding Food Additives

Establishment of Supplement

- January 23, 2017: 158th meeting of the Food Additives Expert Committee
March 7, 2017: 641st meeting of FSCJ (report by the Food Additive Expert Committee meeting)
March 8 to April 6, 2017: Public comments
July 12, 2017: Report to the Chairperson of FSCJ by the Chairperson of the Food Additive Expert Committee
July 18, 2017: 654th meeting of FSCJ (report by the Food Additive Expert Committee meeting)

Amendment associated with establishment of other guidelines

- July 18, 2017: 654th meeting of FSCJ (deliberation)
Adopted and published as the amended Guidelines for Assessment of the Effect of Food on Human Health Regarding Food Additives

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Chapter I. 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, January 16, 2004)¹.

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).

Guidelines for 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.

The FSCJ has established “Guidelines for Assessment of the Effect of Food on Human Health Regarding Food Additives”, May 2010, based on the results of the previously conducted risk assessments of additives as well as approaches for the risk assessments in Japan and other countries. Since then, these guidelines have been applied for the risk assessment of additives.

Thereafter, FSCJ established each of Guidelines for the Assessment of Flavoring Substances in Foods on Health (May 17, 2016), Guidelines for the Risk Assessment of Additives (Enzymes) in Food (July 18, 2017) and Guidelines for the Risk Assessment of Food Additives for Fortification (July 18, 2017). Consequently, the present guidelines shall be applied for the risk assessments of the additives other than flavoring substances, enzymes and food additives for fortification, from now on.

In addition, FSCJ summarized Approaches for the Risk Assessment of Processing Aids (Food Disinfectants and Extractants) as the Supplement to the present guidelines.

¹ "Basic Matters" (the Cabinet Decision, January 16, 2004) has been replaced by "Basic Matters" (the Cabinet Decision, June 29, 2012).

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. Definition

1. Food additives

Food additives are defined in Article 4-2 of the Food Sanitation Act (Law No. 233 of 1947) as substances which 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.

2. ADI: Acceptable daily intake

The ADI is a measure of the amount of a specific substance that is ingestible on a daily basis over a lifetime assumingly without an adverse health effect based on the current scientific knowledge.

3. UL: Tolerable upper intake level

The UL refers to the maximum level of the long-term average daily intake from all sources judged to be unlikely to lead to adverse health effects in humans.

4. NOAEL: No-observed-adverse-effect level

The NOAEL is the highest dose of a substance that causes no detectable adverse effect, found by toxicity study under defined conditions of exposure.

5. LOAEL: Lowest-observed-adverse-effect level

The LOAEL is the lowest dose of a substance that causes a detectable adverse effect, found by toxicity study under defined conditions of exposure.

6. BMD: Benchmark dose

The BMD is a dose level that is estimated from the fitted dose-response curve, associated with a specified change in response, by applying the statistical model.

7. VSD: Virtually safe dose

The VSD is a dose of a substance at which the risk of cancer is no greater than normal if the food containing the substance were consumed at the maximum residue level over a lifetime (i.e., low probabilities, such as 1/100,000 or 1/1,000,000). This dose is used for assessment methods based on the concept that there is no threshold for genotoxic-carcinogenic substances.

8. Endpoint

Endpoint is an observable or measurable biological event and chemical concentration etc. that are used as an indicator of exposure effects of the target substance.

9. Safety factor

Safety factor is a factor used to consider further safety in calculating ADI etc. from NOAEL or LOAEL for a specific substance.

10. MOA: Mode of action

The MOA refers to the mechanism by which a chemical substance affects organisms.

11. WOE: Assessment based on the weight of evidence

The WOE refers to an assessment based on the weight of the evidence examined.

12. GLP: Good laboratory practice

The GLP refers to standards for quality of practice and equipment of a testing institution, as well as its organization, staff and operational procedures. The aim is to ensure the reliability of results of safety tests of various chemical substances.

13. Epidemiology

A field of study to reveal the incidence, distribution of health related issues that occur in human populations, and the influencing factors such as dietary, smoking and drinking habits, aiming to serve for establishment of effective measures for the health related issues.

14. Joint FAO/WHO Expert Committee on Food Additives (JECFA)

A joint committee of FAO and WHO to conduct risk assessments for food additives, contaminants, veterinary products, and other items, and to provide scientific recommendations to member states and the Codex Alimentarius Commission.

15. The 1996 Guidelines by the Ministry of Health and Welfare

“Concerning the Guidelines for Designation of Food Additives and for Revision of Standards for Use of Food Additives” (Notification No. 29 of March 22, 1996)

16. Internationally commonly used food additives

Internationally commonly used food additives are food additives designated based on the agreement reached by the Food Sanitation Subcouncil of the Pharmaceutical Affairs and Food Sanitation Council on July 2002 for which (1) an international safety assessment has been completed by the JECFA and (2) the use has been widely permitted in the U.S. and EU member states and there is a global consensus on the necessity of use. The Ministry of Health, Labour and Welfare of Japan has indicated a policy to start reviewing substances for this designation without waiting for requests to be made by private corporations and other organizations.

Article 3. Purpose

The purpose of the guidelines is to establish the guiding principle of risk assessment on additives and to define the scope of the required documents. The risk

assessment shall 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.

Assessments must be conducted following these guidelines when specific names of additives are removed from the existing list of additives based on the Supplementary Provision Article 2-2, paragraph (1) of the Act for Amendment of the Food Sanitation Act and the Nutrition Improvement Act (Law No. 101 of 1995).

Article 4. Approach for the Risk Assessment of Food Additives

1. Safety factors will be applied to the risk assessments of the additives after FSCJ finalizes the approach. For the time being, the Expert Committee shall have the responsibility of deciding how to apply safety factors
2. For the additives which have gone through the risk assessment by JECFA and have already been used in the U.S. and Europe for a long time, namely “Internationally commonly used food additives”, assessment based on assessment reports by the JECFA, the U.S. and European countries (“report-based assessments”) shall be conducted in principle after the latest scientific findings are examined.
3. Existence of a threshold of genotoxic carcinogens has been debated internationally, but consensus is yet to be reached regarding this matter. Assessment, therefore, shall be conducted in principle based on the concept that no such threshold exists. Any examination for specifying a substance as a genotoxic carcinogen must be conducted carefully considering the MOA and WOE.
4. In principle, food additives that are assessed and determined to be genotoxic carcinogens should not be approved in accordance with the previous paragraph at this point. If the substance is a contaminant (including naturally occurring component; the same hereinafter) that is unavoidably added during the manufacturing process of the food additive, or if a byproduct of the substance is a genotoxic carcinogen, assessment shall be comprehensively conducted based on the concept of VSD, while the content level should be lowered to the minimum that is technologically possible.
5. If data suggesting potential risks are available, the risk assessment in pregnant women, fetuses, infants, children, and the elderly shall be conducted as necessary.
6. 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 additives is desirably to be considered, if needed. For example, the *in-vitro* data

obtained using human metabolic enzymes may be extrapolated to humans in case the adverse effects from the metabolites are concerned in animal studies.

7. In case the target additive is considered to have a potential to interact with a pharmaceutical, such interaction in principle should occur in person under the care of healthcare professionals. Therefore, the related issues shall be examined if information to suggest risk from such interaction is available.
8. Concerning degradation products of the additives, mixed impurities and human specific metabolites, necessity of the evaluation shall be examined. Stability of the additives, including stability in food, shall be also examined. If not stable, types and amounts of major degradation products shall be characterized.
9. Safety of combined intake of multiple additives 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 by FSCJ. When there is any finding of risk from combined intake of multiple additives, however, safety shall be evaluated as necessary.
10. 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.
11. According to JECFA, food additives derived from a novel technologies such as nano-materials may have toxicological properties different from those of the conventional additives, and therefore the existing specifications and the ADI cannot be applied to these substances in general. When assessment of these substances becomes necessary, each case shall be examined appropriately.

Article 5. Approach for the Documents Required

1. The scope and the points to be considered regarding the documents required for assessments are shown in Chapter II and Appendix, and additional information is given below. Practical procedures of each study are recommended to follow the test guidelines that are approved internationally, such as those of the Organization for Economic Co-operation and Development (OECD).
 - (1) Part of the studies can be omitted when it is scientifically revealed that the additive is a common ingredient of food or becomes a common component of food after the additive is broken down in the food or in the gastro-intestinal tract. The scientific validity of such information shall be determined after examination of the items in Table 2 of the 1996 Guidelines by the Ministry of Health and Welfare.

- (2) Assessments of “Internationally commonly used food additives” should be conducted taking into consideration the long history and experience of human dietary habits (see Chapter I, Article 4-2).
 - (3) When the target additive differs from an already designated additive only in the base moiety, when it is an isomer of such a designated additive, or when there are scientific rationales, part of the studies can be omitted with clarifying the rationales.
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.
 - ① For the amendment of standards for use of an additive of which risk assessment by FSCJ is already completed, applicants shall submit documents concerning the estimation of the daily intake of the additive considering requested addition of foods in which the additive is to be used or considering changes in the amount of use of the additive. Even in this case, applicants shall also submit documents regarding new toxicological findings, if any.
 - ② When the risk assessment of the target additive 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 additive.
 3. 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 additive, however, applicants shall provide the data irrespective of the reliability because such information may be necessary for evaluating the additive.
 4. FSCJ recommends performing autopsy and histopathological evaluation by experienced experts.
 5. 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. Evaluation in principle shall be conducted using the documents submitted by the applicant. If the documents are considered insufficient for evaluation, the applicant is asked for additional documents.

Article 6. Interpretation of toxicokinetics and toxicity study

Study of toxicokinetics is for assuming biological fate of ingested target additives, such as absorption, distribution, metabolism in human body, and excretion. Therefore besides summarizing the animal data, the fate within the human body and the possible occurrence of adverse health effects should be discussed.

In interpreting the data, it should be scientifically clarified that the observed toxicity and residual property in the body are the intrinsic characteristics of the additive and not an incidental effect of the extrinsic factors such as the nutritional condition of the subject. Judgment of an endpoint should be made based on the scientific rationale of statistical significance and dose-response relationship in the findings in each toxicity study such as general condition, body weight, food intake, hematological tests, blood biochemistry tests, urine tests, pathological tests and others, besides considering the differences in toxicokinetics, animal species and test doses in each study.

In these cases, the toxicological mechanism should be clarified as much as possible.

Article 7. Risk characterization

1. Approach for establishing ADI

ADI should be established based on the following principle.

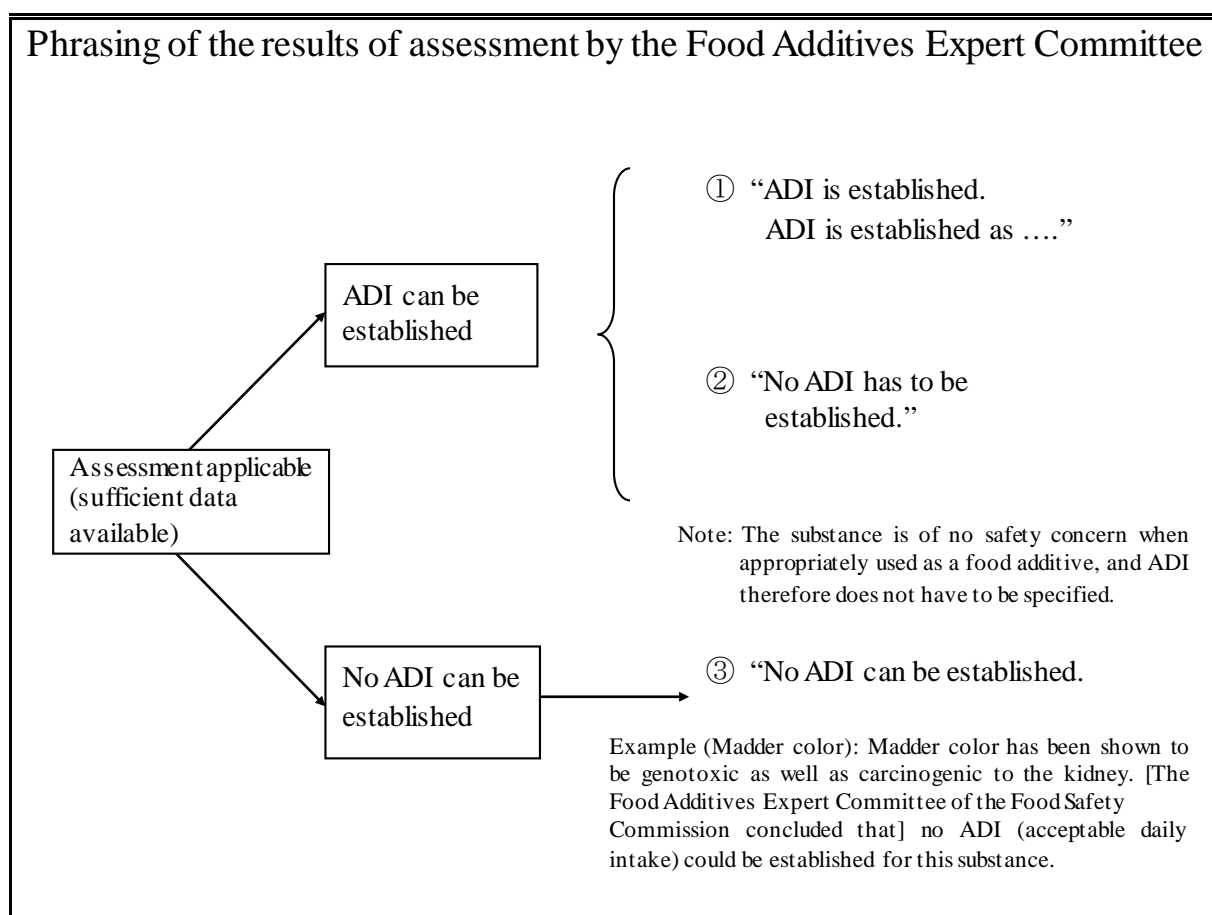
- (1) When more than one NOAEL are determined by comprehensive evaluation of the toxicity studies, the NOAELs shall be compared among each group of studies in terms of type of toxicity and of animal species, then the ADI should be established based on the lowest NOAEL value in principle
- (2) Fundamentally, a safety factor of 100 (10 for species differences, 10 for individual differences) shall be applied for establishing ADI, considering species differences and individual differences. However, the safety factor of 100 is not a fixed constant value but rather should be specified individually in each case based on the type of toxicity and test data as follows.
 - ① When the data are taken from human studies, consideration of species differences is not necessary. Considering individual differences, a safety factor of 1 to 10 shall be applied depending on the surveyed populations.
 - ② In case sufficient information is not available or in case the target additive is associated with serious toxicity², an additional factor of 1 to 10 for each case shall be added to the safety factor.

² The Principles for the Safety Assessment of Food Additives and Contaminants (IPCS, EHC70) lists the following two examples:

- a) Irreversible reaction seen in a prenatal developmental toxicity test
- b) Finding of carcinogenicity

- ③ When the ADI is established based on the LOAEL, an additional factor of 1 to 10 shall be added to the safety factor. A benchmark dose can be also used in these cases.

(3) The phrasing of the assessment result should follow the pattern set out below.



2. Determination of NOAEL

When a no-observed-adverse-effect level (NOAEL) of the target additive 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 additive 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 additive. 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 additive, 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.

3. Group ADI

When several substances that have a structure activity correlation or have similar toxicities without a structure activity correlation that can cause additive physiological/toxicological effects are used as additives, an ADI should be established for the substances as a group in order to manage the accumulated intake. When establishing a group ADI, the lowest NOAEL among the NOAELs of all the substances in the group should be used, in principle. The relative quality of the test data and the test period should be taken into consideration when establishing the NOAEL. If one NOAEL is significantly higher or lower than the other NOAEL values of the substances in the group, that substance should be excluded from the group.

Article 8. Re-evaluation

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

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

Chapter II. Detailed Exposition

The documents required for the assessment are listed in Appendix. For detail, the notes below should be followed.

Article 1. Outline of the Target Additive

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, CAS number), molecular structure, molecular weight, structural formula, manufacturing method, property, stability (including stability in food), 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, based on the comprehensive evaluation of safety and efficacy of the additive, rationales for setting the standards for use need to be clarified. When setting the standards, the results of comparison of the estimated daily intake (refer to Article 4 of Chapter 2,) with the ADI obtained from toxicity studies should be taken into consideration.
 - (2) When setting the standards for use is considered unnecessary, rationales for the consideration need to be clarified.
7. Other (Information useful for the risk assessments)

Article 2. Information relevant to safety

1. Toxicokinetics

Toxicokinetics shall be studied in accordance with the descriptions on toxicokinetics study in the 1996 Guidelines by the Ministry of Health and Welfare, in addition to the following requirements.

- (1) As the test substance, the food additive or its isotope-labeled compound should be used. When an isotope-labeled compound is used, the labeling nuclide and its position should be clearly indicated.
- (2) Animal studies on toxicokinetics shall be preferably conducted in two or more species; one or more species of rodents (generally rats) and one or more species of non-rodents (generally dogs).

- (3) In principle, the test substance should be administered orally. Absorption, distribution, metabolism, and excretion should be estimated after single-dose administration and repeated-dose administration. Additional tests with intravenous administration or other administration may be conducted when necessary for other purpose such as calculation of accurate ratio of absorption.
- (4) Examination of each process of absorption, distribution, metabolism and excretion requires the following experimental data of the test substance; blood concentration of the active ingredient, the amount excreted in urine, feces and other excretory matter, time course of the concentration in each organ; the metabolites, as well as factors that influence each step.
- (5) Data of animal studies on toxicokinetics of absorption, distribution, metabolism and excretion (e.g., the highest plasma concentration, time course of concentration in each organ, and elimination half-life) shall be used for assuming the potential target organ for the toxicity studies. In such cases, it shall be examined whether the animal data can be extrapolated to human considering the species difference and species specificity.
- (6) When the test substance is a racemate, it is desirable to examine the toxicokinetics of each optical isomer as necessary in relation to the toxicity.
- (7) In principle, the existence of human-specific metabolites must be examined and toxicity studies of such metabolites must be conducted as necessary.

2. Toxicity study

(1) Subchronic toxicity study and chronic toxicity study

- ① Studies should be conducted in one species of rodent (generally rats) and one species of non-rodent (generally dogs). In principle, the same number of male and female animals should be used.
- ② The administration period should be 28 days or 90 days for subchronic toxicity studies and more than 12 months for chronic toxicity studies. Study with administration for 28 days can be omitted when a study with a 90-day administration period is conducted.
- ③ In principle, the test substance should be orally administered 7 days a week. The substance should be administered by feeding or in drinking water, but it may also be administered by gavage when the administration by feeding or in drinking water is practically difficult
- ④ Studies should be conducted in at least three different dose groups in addition to the control group. The reasons for choosing each dose should be clearly indicated, and common ratios should be chosen so that an appropriate NOAEL can be obtained.

- ⑤ In case of a feeding study, care should be taken to prevent nutritional disorder. Generally, a dosage of a feed containing the test substance 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.
- ⑥ In case where a naturally occurring pathological change that is also observed in the control animals shows increases in the frequency or severity with biologically relevant difference such as a dose-response relationship by the administration of the test substance, the increase shall be taken as an effect caused by the administration even if the increase is within the range of background data.
- ⑦ When neurotoxicity or immunotoxicity³ is suspected, the need for additional studies following the OECD test guidelines or ICH (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use) guidelines should be considered as necessary.
- ⑧ When the findings observed in toxicity studies are extrapolated to humans, how to extrapolate the data should be examined for data in each group of endpoint classified by different factors, such as functional changes, non-oncological morphological changes, oncological morphological changes, and changes of reproductivity, for careful interpretation.
- ⑨ When a combined chronic toxicity/carcinogenicity study is conducted in one species of rodent, a chronic toxicity study in the same rodent can be omitted.
- ⑩ The need to add an *in utero* exposure phase should be examined as necessary.

(2) Carcinogenicity study

- ① Studies should be conducted in two or more species of rodent (rats, mice or hamsters are used generally). In principle, the same number of male and female animals should be used.
- ② In principle, the test substance should be orally administered 7 days a week. The administration period should be between 24 months and 30 months for rats, and between 18 months and 24 months for mice. The substance should be administered by feeding or in drinking water, but it

³ In this guideline, “immunotoxicity” is defined as toxicity resulting from suppressed immune function caused by a substance unintentionally ingested by a living organism in a non-antigen-specific way.

may be also administered by gavage when the administration by feeding or in drinking water is practically difficult.

- ③ Studies should be conducted in at least three different dose groups in addition to the control group. The reasons for choosing each dose should be clearly indicated, and common ratios should be chosen so that an appropriate NOAEL can be obtained.
- ④ In case of a feeding study, care should be taken to prevent nutritional disorder. Generally, a dosage of a feed containing the test substance 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.
- ⑤ If an additive is judged as genotoxic-carcinogenic because the carcinogenicity is positive and the genotoxicity is also positive, the ADI cannot be established in principle. If the genotoxicity is negative and the additive is clearly judged to be a non-genotoxic carcinogen, the ADI can be established. Even if the target additive unavoidably generates/contains a byproduct/residue suspected of genotoxicity, the ADI may be established in some cases after a required examination (refer to Chapter I, Article 4-3, 4-4).
- ⑥ In case the incidence rate of lesions is relatively low, carcinogenicity of the target additive may be judged by conducting a significance test among the incidence numbers using either: (1) the sum of benign tumor-like lesions and malignant tumor-like lesions; or (2) the sum of precancerous lesions, benign tumor-like lesions and malignant tumor-like lesions. Especially for increasing incidence of tumors in the endocrine system that frequently occur in rodents, the carcinogenicity should be desirably assessed including precancerous lesions.
- ⑦ When an increase in incidence of tumors is detected in a region where the incidence is not normally high or when an increase in incidence of rare tumors is detected, it is desirable that the carcinogenic mechanism is also discussed in the assessment.
- ⑧ Factors that modify incidence of cancer (suppression of body weight gain or decrease of survival rate) should be taken into consideration for the assessment.
- ⑨ Toxicological findings specific to the species such as hypertrophy, hyperplasia and tumor of thyroid follicle epithelium in rodents, and renal disorder and tumor in male rats should be taken into consideration.
- ⑩ When a combined chronic toxicity/carcinogenicity study is carried out in

one species of rodent, a carcinogenicity study in of the same rodent can be omitted.

- ⑪ The need to add an *in utero* exposure phase should be examined as necessary.

(3) Combined one-year repeated dose toxicity/carcinogenicity study

Points to be considered in (1) and (2) should be followed.

(4) Reproductive toxicity study

Study shall be conducted in accordance with the descriptions on reproductive toxicity study in the 1996 Guidelines by the Ministry of Health and Welfare, in addition to the following requirements in addition to the following requirements.

- ① Studies should be conducted in one or more species of rodent (rats are used generally). In principle, the same number of male and female animals should be used.
- ② In principle, the test substance should be orally administered 7 days a week. The substance should be administered by feeding or in drinking water, but it may be also administered by gavage when the administration by feeding or in drinking water is practically difficult.
- ③ Studies should be conducted in at least three different dose groups in addition to the control group. The reasons for choosing each dose should be clearly indicated, and common ratios should be chosen so that an appropriate NOAEL can be obtained.
- ④ In the case of a feeding study, care should be taken to prevent nutritional disorder. Generally, a dosage of a feed containing the test substance 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.
- ⑤ When neurotoxicity or immunotoxicity is suspected, the need for additional studies as described in the OECD test guidelines or ICH guidelines should be examined.

(5) Prenatal developmental toxicity study

Study on prenatal developmental toxicity shall be conducted in accordance with the description of teratogenicity study in the 1996 Guidelines by the Ministry of Health and Welfare. The substance shall be administered daily to

the pregnant animals for the period at least from the date of implantation to the day before the expected delivery date.

- ① Studies should be conducted in two or more species in total, that is, one or more rodent [generally rats] and one non-rodent [generally rabbits].
- ② The test substance should be orally administered by gavage.
- ③ Studies should be conducted in at least three different dose groups in addition to the control group. The reasons for choosing each dose should be clearly indicated, and common ratios should be chosen so that an appropriate NOAEL can be obtained.

(6) Genotoxicity study

Although studies follow the description of mutagenicity study in the 1996 Guidelines by the Ministry of Health and Welfare, the studies should not be limited to “mutagenicity” of narrow definition and the assessment should be conducted based on the examination of the genotoxicity in general. Among the studies composing the standard combination (i.e., combination of reverse mutation tests using bacteria, chromosomal aberration tests in cultured mammalian cells, and micronucleus tests in rodents), chromosomal aberration tests in cultured mammalian cells can be substituted with a mouse lymphoma TK assay (MLA) or *in vitro* micronucleus test. As additional studies to supplement the results from studies of the standard combination, single cell gel electrophoresis (“Comet Assay”) and *in vivo* transgenic animal mutation assay may be used, in addition to those described in the 1996 Guidelines by the Ministry of Health and Welfare.

When one of the studies in the standard combination cannot be conducted due to the technical constraints, the study can be substituted with a study of which validity for the substitution has been internationally verified, in condition that the reason of the constraints is explained based on the scientific evidence.

The results should be judged in accordance with the following procedure.

- ① If the results of the reverse mutation tests using bacteria are positive, the genotoxicity should be assessed comprehensively by fully considering the results of *in vivo* tests that use genetic mutation or DNA damage as an indicator (comet assay, *in vivo* transgenic animal mutation assay).
- ② If the results of the chromosome aberration tests using cultured mammalian cells are positive and the effect is also confirmed with rodent micronucleus tests, the substance can be determined as positive for genotoxicity.
- ③ If the results of micronucleus tests in rodents appropriately conducted with up to high doses (preferably with evidence to show exposure of the target

organ) are negative despite the positive results of the chromosome aberration tests using cultured mammalian cells, genotoxicity of the substance can be negated.

(7) Allergenicity⁴ study

Studies to examine the allergenicity of food additives should be conducted in accordance with the description of antigenicity study in the 1996 Guidelines by the Ministry of Health and Welfare. No method has been well-established yet for predicting the allergenicity of orally ingested chemical substances, particularly for predicting the immediate type of allergenicity. Therefore, allergenicity of the additives should be studied with sensitization and induction methods approved by specialists. For the time being, allergenicity studies using delayed allergy as an indicator should at least be conducted. Examples of tests for such studies include skin sensitization tests on guinea pigs (e.g., guinea pig maximization test [GPMT] in the OECD test guideline 406) and lymph node reaction tests on mice (e.g., the local lymph node assay [LLNA] in the OECD test guideline 429).

Allergenicity assessment of food additives containing protein as the constituent should follow the “Standards for the Safety Assessment of Genetically Modified Foods (Microorganisms)” (FSCJ decision, June 26, 2008).

(8) General pharmacological study

General pharmacology of food additives should be studied in accordance with the description of general pharmacological study in the 1996 Guidelines by the Ministry of Health and Welfare.

(9) Other studies

When neurotoxicity is suspected in a subchronic toxicity study and other studies, additional studies should be conducted as necessary following guidelines such as the OECD test guideline.

When immunotoxicity is suspected in a subchronic toxicity study and other studies, proper immune function test should be added as necessary in accordance with the ICH guideline. Immune function test should be also conducted as necessary when immunotoxicity in humans is suspected based on existing findings.

Article 3. Findings in humans

⁴ It is also called allergy-inducing effects.

When available, appropriate clinical tests, epidemiological data and other information regarding humans should be utilized. When allergenicity is suspected, findings in humans should be especially valued because it is often infeasible to extrapolate the animal data to humans.

Article 4. Estimation of daily intake

1. Daily intake in Japan shall be estimated, with care for avoiding underestimation, as follows. The daily intake of the additives in the food shall be estimated in principle multiplying the daily intake of the subject food product⁵ by the concentration of the additive. 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 documents. For daily intake of additives, estimation may also be conducted based on the data obtained by reliable methods such as market basket investigation or survey based on production statistics. Body weight used for the estimation shall be the average body weight designated in the latest decision of FSCJ.
2. The estimated daily intake should be compared with the ADI obtained from toxicity studies, and the results of the comparison should be discussed in the assessment. The safety of food additives should also be evaluated as necessary in case of simultaneous consumption of the same kinds of additives by comparing the sum of estimated daily intake of each additive to the group ADI.
3. Effects of food additives on the overconsumption of nutrients and on electrolyte balance should also be evaluated, as necessary, based on the actual situation of food consumption in Japan.

Article 5. Assessment of Flavoring Substances

Assessments of flavors follows “Guidelines for the Assessment of Flavoring Substances in Foods on Health”⁶.

Article 6. Assessment of Enzymes

Assessment of enzymes follows “Guidelines for the Risk Assessment of Additives (Enzymes) in Foods”⁷.

Article 7. Assessment Food Additives for Fortification

⁵ The food product in which the additive is to be used.

⁶ FSCJ decision, May 17, 2016

⁷ FSCJ decision, July 18, 2017

Assessment of food additives for fortification follows “Guidelines for the Risk Assessment of Food Additives for Fortification”⁸.

⁸ FSCJ decision, July 18, 2017

Appendix. Documents required for the target additive

| Items | Designation | Revision of standard |
|--|-------------|----------------------|
| Outline of the target additive | | |
| 1. Name and usage | Required | Required |
| 2. Origin or history of discovery | Required | * |
| 3. Usage in other countries | Required | Required |
| 4. Risk assessments by international organizations and other organizations | Required | * |
| 5. Physicochemical properties | Required | * |
| 6. Draft standards for use | Required | Required |
| 7. Other (Information useful for the risk assessment) | * | * |
| Information relevant to safety | | |
| 1. Toxicokinetics study | Required | * |
| 2. Toxicity | | |
| (1) Subchronic toxicity study and chronic toxicity study | Required | * |
| (2) Carcinogenicity study | Required | * |
| (3) Combined one-year repeated dose toxicity/carcinogenicity study | Required | * |
| (4) Reproductive toxicity study | Required | * |
| (5) Prenatal developmental toxicity study | Required | * |
| (6) Genotoxicity study | Required | * |
| (7) Allergenicity study | Required | * |
| (8) General pharmacological study | Required | * |
| (9) Other studies | * | * |
| 3. Findings in humans | Required | * |
| 4. Estimation of daily intake, etc. | Required | Required |
| | | |

Note 1. When requesting an amendment of the standards for use of a food additive of which the risk assessment by FSCJ has already been completed, the documents required for “Revision of standards” should be submitted. For a food additive of which the risk assessment by FSCJ has not been conducted, documents required for designation should be submitted, in principle.

Note 2. Documents marked “Required” shall be submitted. Documents marked with an asterisk (*) should be submitted as necessary (when there is a new finding, for example).

Note 3. When a combined one-year repeated dose toxicity/carcinogenicity study is carried out using one rodent species, a chronic toxicity study and carcinogenicity study on the same rodent species can be omitted.