Guideline for Assessment of the Effect of Food on Human Health Regarding Food Additives

May 2010

Food Safety Commission of Japan (FSCJ)

Table of Contents

<History of Evaluation>

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 Pesticides Expert Committee meeting)
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 Chairman of FSCJ by the Chairman of the Pesticides Expert Committee
May 27, 2010: 333rd meeting of FSCJ (reporting of the discussion results from the Pesticides Expert Committee meeting)
Adapted and published as the Guideline for Assessment of the Effect of Food on Human Health Regarding Food Additives

<Food Safety Commission Members>

(Until June 30, 2009)	(From July 1, 2009)
Dr. Takeshi Mikami (Chair)	Dr. Naoko Koizumo (Chair)
Dr. Naoko Koizumi (Deputy Chair)	Dr. Takeshi Mikami (Deputy Chair*)
Dr. Taku Nagao	Dr. Taku Nagao
Dr. Kazumasa Nomura	Dr. Kazumasa Nomura
Dr. Keiko Hatae	Dr. Keiko Hatae
Dr. Masao Hirose	Dr. Masao Hirose
Dr. Seiichi Honma	Dr. Masatsune Murata
	* From July 9, 2009

<Food Safety Commission Food Additives Expert Committee Members>

(Until September 30, 2009)	(From October 1, 2009)
Dr. Shoji Fukushima (Chairman)	Dr. Katsumi Imaida (Chairman)
Dr. Yasushi Yamazoe (Deputy Chairman)	Dr. Yasushi Yamazoe (Deputy Chairman)
Dr. Mayumi Ishizuka	Dr. Mayumi Ishizuka
Dr. Kazuhide Inoue	Dr. Kiyomi Ito
Dr. Katsumi Imaida	Dr. Kazuhide Inoue
Dr. Takashi Umemura	Dr. Takashi Umemura
Dr. Makoto Ema	Dr. Makoto Ema
Dr. Kikue Kubota	Dr. Kikue Kubota
Dr. Masahiro Tokin	Dr. Tetsuya Tsukamoto
Dr. Dai Nakae	Dr. Masahiro Tokin
Dr. Emi Nakajima	Dr. Dai Nakae
Dr. Makoto Hayashi	Dr. Makoto Hayasi
Dr. Kunitoshi Mitsumori	Dr. Kunitoshi Mitsumori
Dr. Nobuo Yoshiike	Dr. Akemi Morita
	Dr. Masami Yamada

[Adviser]
Dr. Kiyomi Ito
Dr. Junichi Sawada
Dr. Kentaro Tanemura
Dr. Tetsuya Tsukamoto
Dr. Masato Naya
Dr. Masamichi Honma
Dr. Akemi Morita
Dr. Masami Yamada

Chapter I. General Provisions

Article 1. Background

The Food Safety Commission of Japan (FSCJ), based on the "Basic Matters" referred to in Article 21-1 of the Food Safety Basic Law (approved by the Cabinet on January 16, 2004), is obliged to establish guidelines for assessing the effect of foods on human health. The following guidelines have been established in accordance with this obligation: "Standards for the Safety Assessment of Genetically Modified Foods (Seed Plants)" (January 29, 2004), "Guideline for Assessment of the Effect of Food on Health Regarding Official Standards of General Fertilizers" (March 18, 2004), "Standards for the Safety Assessment of Food Additives Produced Using Genetically Modified Microorganisms" (March 25, 2004), "Guideline for Safety Assessment of Genetically Modified Animal Feed and Animal Feed Additives" (May 6, 2004), "Guideline for Assessment of the Effect of Food on Human Health Regarding Antimicrobial-Resistant Bacteria Selected by Antimicrobial Use in Food Animals" (September 30, 2004), and "Standards for the Safety Assessment of Genetically Modified Foods (Microorganisms)" (June 26, 2008).

Guidelines for the assessment of the effect of food on human health are essential for ensuring the scientific validity and fairness of assessments as well as clearly defining the assessment data for applicants. They also serve to assure parties both within and outside Japan of the transparency of assessments.

FSCJ of Japan has created this "Guideline for Assessment of the Effect of Food on Human Health Regarding Food Additives" based on the results of past assessments of the effect of food on health as well as policies regarding safety assessments developed by the Japanese government and governments of other countries. Going forward, this should be followed whenever assessments are conducted.

It should be noted that this Guideline for Assessment will be reviewed in accordance with regulations as necessary in consideration of trends in international standards for assessments and new scientific findings both in and outside Japan. Based on such reviews, the Guideline may be revised.

Article 2. Definition

1. Food additives

   As defined in Article 4-2 of the Food Sanitation Act (Law No. 233 of 1947), 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. Acceptable daily intake (ADI)

   Based on contemporaneous scientific knowledge, the amount of a substance that can be ingested on a daily basis over a lifetime without appreciable health risk to the consumer.

3. Tolerable upper intake level (UL)

   The maximum level of habitual nutrient intake that is likely to pose no risk of adverse effects.

4. No-observed-adverse-effect level (NOAEL)

   The highest dose of a substance that has been reported to have no harmful effects in toxicological tests involving different dosage levels.

5. Lowest-observed-adverse-effect level (LOAEL)

   The lowest does of a substance that has been reported to have harmful effects in toxicological tests involving different dosage levels.

6. Benchmark dose (BMD)

   The intake level at a certain toxicological response rate calculated by applying a mathematical model to the correlation between the toxicological response rate and the intake.

7. Virtually safe dose (VSD)

   The 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 assumption that there is no threshold for genotoxicological substances.

8. Toxicological indicator (Endpoint)

   The observable or measurable biological incidence or chemical concentration that is used as an indicator of exposure effects of the assessed substance.

9. Safety factor

   The factor used to translate an NOAEL when setting the ADI and other levels to ensure further safety.

10. Mode of action (MOA)

    The mechanism by which the chemical substance affects organisms.

11. Assessment based on the weight of evidence (WOE)

    An assessment based on the weight of the evidence examined.

12. Good laboratory practice (GLP)

    Standards of quality of practice at the testing facilities and equipment of a testing institution, as well as its organization, staff and operational procedures. Set to ensure the reliability of results of safety tests of various chemical substances.

13. Epidemiology

A field of study in which the incidence and distribution—and factors that influence these, such as dietary, smoking and drinking habits—of various health problems occurring in human populations is examined in order to establish effective measures for issues relating to human health.

14. Joint FAO/WHO Expert Committee on Food Additives (JECFA)
    A committee jointly organized by FAO and WHO to conduct risk assessments for food additives, pollutants, veterinary products, and other items, and provide recommendations grounded in scientific findings to member states and the Codex Alimentarius Commission.
15. The 1996 Guideline 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
    Food additives so designated based on the agreement reached by the Food Sanitation Subcommittee 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 this Guideline is to define the scope of data required to assess effects and the guidelines for assessment to be used, when additives are sought to be designated as not harmful for human health based on Article 10 of the Food Safety Law and, to define the standards for additives based on Article 11-1 of the same Law.

Assessments must be conducted following this Guideline when specific names of additives are removed from the existing list of additives based on the Supplementary Provision 2-2-1 of the Act for Partial Revision of the Food Sanitation Act and the Nutrition Improvement Act (Law No. 101 of 1995).

Article 4. Policies for assessments of the effect of food on health regarding food additives

1. Safety factors will be applied to the values determined as a result of assessments of the effect of food on health regarding food additives after FSCJ finalizes the treatment. For the time being, the Expert Committee shall have the responsibility of deciding how to treat the safety factors.

2. For “Internationally commonly used food additives” which are broadly used internationally and proven safe (excluding internationally commonly used flavors), which have passed the safety assessment conducted by the JECFA and are approved for long-term use in the U.S. and Europe, assessments based on assessment reports generated 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. Although the existence of a threshold for genotoxic carcinogens[1] has been a topic of international discussion for some time, no consensus has yet been reached. Assessment, therefore, shall be conducted in principle based on the assumption that no such threshold exists. Any examination of a substance for classification as a genotoxic carcinogen must be conducted carefully and conclusions must based on the MOA and WOE.
4. In principle, food additives that are assessed and determined to be genotoxic carcinogens should not be approved at this point in accordance with the previous paragraph. If the substance is foreign matter (including naturally occurring matter; 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. Substances that are to be used as an alternative to ordinary food ingredients or for the purpose of nutritional enhancement or as a “food with nutritional claims,” must be examined regarding their quality as a nutritional component as well as relative to the intake levels of the same nutritional components that are available in other foods. Dietary Reference Intakes and other information must also be examined in order to make an assessment.
6. Effects on pregnant women and fetuses, infants, children and elderly people should be examined as necessary in cases where sufficient information is available to examine risks.
7. It is preferable to examine as necessary *in vitro* studies and other studies conducted during pharmaceutical development or in other areas that are recommended for food additive studies (for example, when a metabolite has had a harmful reaction in an animal test, the results must be extrapolated to effects on humans by conducting an *in vitro* test using a human metabolic enzyme).
8. When interaction between the assessed substance and a medical product is likely to occur, the interaction shall be examined only when necessary and when sufficient knowledge is

[1] A genotoxic carcinogen is a substance that affects DNA either directly or by its metabolites to show inducibility for genetic mutation or chromosomal aberration, and whose genotoxic reaction is considered a part of the carcinogenic mechanism. Its genotoxicity must be confirmed *in vivo* (in the carcinogenic target organ, if possible).

available to examine the risk. It should be noted that people subject to such reactions are, in principle, under the care of healthcare professionals.

9. The necessity for assessment should be examined also for decomposition products, mixed foreign matter, and metabolites that characteristically effect humans. The safety of food additives and their safe inclusion in food should be also examined and, if found to be unstable, the main types of decomposition products and the levels at which they are generated should also be examined.
10. To examine the harmful effects of intake of more than one food additive, it is considered that the effects of simultaneous intake of different food additives are examined and practical safety can be assured through complete assessment of each food additive based on the reports contained in the "Collection of Information and Survey on Effects of Intake of Multiple Food Additives" compiled in the 2006 General Survey to Ensure Food Safety conducted by FSCJ. However, when findings are unavailable with respect to the risk of taking multiple food additives, assessment should be carried out as necessary.
11. Tests using genetically modified animals call for careful treatment. These tests are rarely used by the JECFA and other organizations except in a very few cases where they are used in risk assessments by FSCJ.
12. The JECFA considers it possible that food additives manufactured with nano materials or other new technologies may have different toxicological features and therefore the existing standards and the ADI cannot be applied to these substances in general. When assessment of these substances becomes necessary, each case will be examined separately.

Article 5. Policies on materials needed for assessments

1. The scope and notes on the data needed for assessments are shown in Chapter II and Appendix 1 and 2, and additional information is given below. Specific test procedures in principle follow the internationally recognized test guidelines published by the Organization for Economic Co-operation and Development (OECD) and other organizations.
   (1) Part of the testing can be omitted when the test food additive is known to be a common ingredient of food or when the test food additive is scientifically known to become a common component of food after the food is broken down in the digestive tract following consumption. The scientific validity of such information shall be determined after examination of the items in Table 2 of the 1996 Guideline by the Ministry of Health and Welfare.
   (2) Assessments of "Internationally commonly used food additives" should take the long history and experience of human dietary habits into consideration (see Chapter I,

Article 4-2) and assessments of “Internationally commonly used” flavors, enzymes or nutritional components should take the characteristics of the substance into consideration (see Chapter II, Articles 5, 6 and 7).

(3) When a test food additive differs from a food additive already designated only in the base moiety, when it is an isomer of such additive, or when there is a scientifically rational reason for doing so, part of the testing can be omitted provided the reason for doing so is clearly described.

2. Revision of usage standards and compositional standards should be conducted in accordance with the notes below.

(1) Revision of usage standards should be done in accordance with the following notes.

(a) When an assessment of the effect of food on health by FSCJ has been completed for the food additive in question, data for the estimated daily intake regarding the request (addition of food items for which the additive is to be used or change of usage dose) should be submitted. If there is a new toxicological finding in such a case, the data on that finding should be also submitted.

(b) When an assessment of the effect of food on health by FSCJ has not been conducted for the food additive in question, materials needed for the assessment for the designation of additives should be submitted, in principle.

(2) For the revision of compositional standards, the validity and safety of the revised compositional standards should be demonstrated.

3. The applicant is responsible for submitting the materials required for the assessment and must ensure the reliability of the content of the materials. In principle, the materials submitted by the applicant must be: (1) results from tests conducted at an experimental facility whose operational management is recognized as appropriate (i.e., a GLP facility) and by a method whose reliability is ensured; or (2) scientifically reliable materials, such as assessment reports complied by international organizations. If materials indicating that there is a concern regarding the safety of the food additive, such materials must be submitted for examination regardless of their reliability.

4. Autopsy and histopathological assessments should be conducted by a specialist with ample experience.

5. Raw data and samples used for animal tests should be maintained until the end of the period regulated under GLP or until the assessment is completed so that they can be submitted if needed.

6. In principle, assessments should be conducted based on the materials submitted by the requesting party. If the materials submitted are considered insufficient, the requesting party may be asked to submit additional materials.

Article 6. Disposition tests and toxicological tests

Disposition tests are conducted to estimate the behaviors of food substances within the human body in terms of absorption, distribution, metabolism, and excretion (ADME). Therefore not only compiling the result of animal tests, ADME behaviors within the human body and the possible occurrence of harmful effects should be examined.

When examining the test data, the observed toxicity and residual level in the body should be confirmed from a scientific point of view to ensure that it is a property of the additive itself and not an incidental effect of other factors such as the nutritional condition of the subject. When deciding an endpoint, the disposition within the body and differences among tests and test animal species should be considered, and the findings in terms of general conditions, body weight, food intake, hematological tests, blood biochemical tests, urine tests, pathological tests and other tests should be examined for statistical significance and dose relations in order to ensure scientifically rational assessment. In these cases, the toxicological mechanism should be determined as clearly as possible.

Article 7. Risk characterization

1. Setting ADI

(1) When more than one NOAEL is indicated as a result of comprehensive assessment of toxicological tests, the ADI should be set based on the lowest NOAEL value.

(2) In principle, results of toxicological tests should be examined by taking sexual differences into consideration, and separate NOAELs should be set for each sex.

(3) Taking species differences and individual differences into consideration, a safety factor of 100 should be used (10 for species differences, 10 for individual differences). It should be noted, however, that the safety factor of 100 is not a fixed, constant value but rather should be set individually in each case based on the toxicological property and test data and in consideration of the following.

(a) When the data are taken from tests on human subjects, species differences do not have to be taken into consideration. Based on individual differences, a safety factor of 1 to 10 should be used, depending on the surveyed populations.

(b) When sufficient information is not available and if the food additive under assessment is associated with serious toxicity[2], the safety factor should be multiplied by an additional value of 1 to 10.

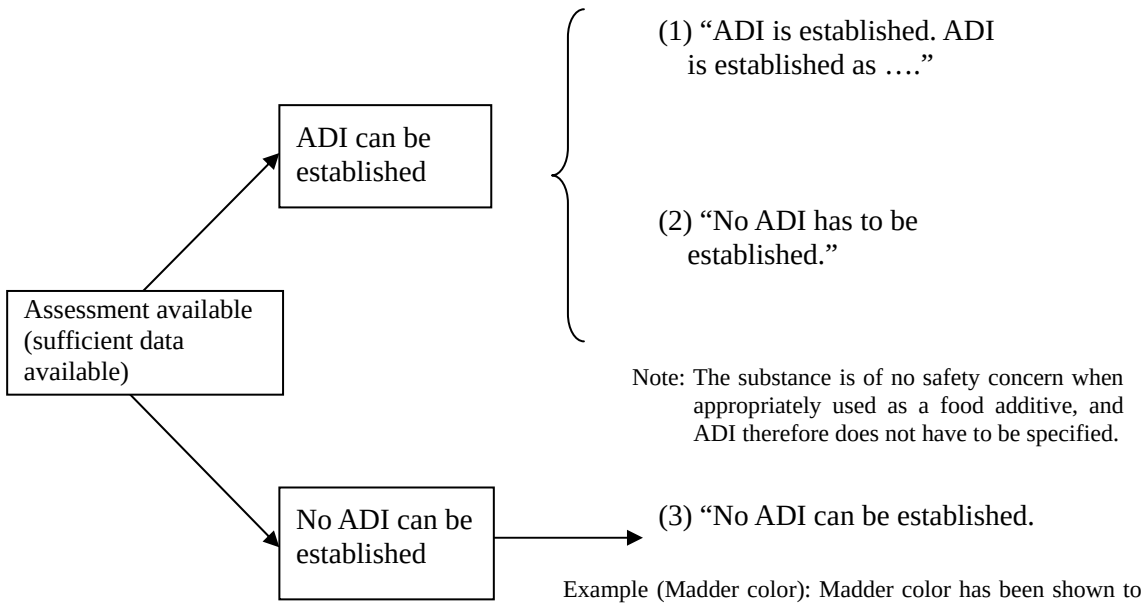
(c) When the ADI is set based on the LOAEL, the safety factor should be multiplied by an additional value of 1 to 10. A benchmark dose can be also used in these

[2] The Principles for the Safety Assessment of Food Additives and Contaminants (IPCS, EHC70) lists the following two items as examples:
a) Irreversible reaction seen in a prenatal developmental toxicity test
b) Finding of carcinogenicity

cases.

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

Phrasing of the results of assessment by the Food Additives Expert Committee



2. Determination of NOAEL

In order to determine the NOAEL, the prior establishment of an appropriate dose should be investigated. In a toxicological test, the maximum dose should be set at the level at which a toxicological effect is recognized, and the minimum dose should be set at the level at which no toxicological effect is recognized. Also, different dose levels should be set so that the dose-reaction relationship can be observed. When the substance is administered by feeding, care should be taken to prevent nutritional disturbance. In general, the ratio of the substance to the feed does not have to exceed 5% (W/W). When the substance is given by gavage administration, the general maximum dose required is the technically possible maximum dose or 1,000 mg/kg bw. If no effect is observed at that dose, the administration of a higher dose is not required.

When different tests are conducted for different animal species, different NOAELs should be determined for each test. To calculate the ADI, the NOAEL taken from the animal test with the lowest dose that shows a toxic effect should be used. However, when

a certain test is obviously more appropriate in terms of its design or results than others and the test periods were different among the tests, a test with a longer period and more appropriate design should be given more weight in determining the NOAEL used to calculate the ADI. When metabolic data or pharmacokinetic data are used, the NOAEL used to calculate the ADI can be determined based on the animal species that most resembles human beings.

3. Group ADI

When several substances that have a ~~structurer~~structure activity correlation or do not have a structural activity correlation but have a similar level of toxicology (e.g., those that can generate additive physiological/toxicological reactions), the ADI should be set 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 removed from the group.

Article 8. Reassessment

Even after a food additive is approved, it should be continuously observed for potential harmful effects and if any harmful effect is identified as a result of progress in toxicological understanding or other factors the additive should be reassessed.

When an important set of data indicating a safety concern is newly obtained regarding a food additive that has previously been assessed, reassessment of that additive should be carried out promptly.

Chapter II. Detailed Expositions

The materials needed for assessments are listed in Appendix 1 and 2. For detail, the notes below should be followed.

Article 1. Information on the food additives to be assessed

1. Name and usage
2. Origin or process of discovery
3. Usage in other countries
4. Assessments by international organizations and other organizations
5. Physiochemical properties

   Chemical name, (generic names in Japanese and English, CAS number), molecular structure, molecular weight, structural formula, manufacturing method, chemical nature, stability (including stability in food), suggestions for compositional standards, etc.
6. Suggestions for usage standards
   (1) When the establishment of usage standards to regulate usage, such as the food items for which the additive can be used and the maximum amount of additive to be used, is considered necessary based on a comprehensive examination of the safety and efficacy of the food additive, the reasons for setting such usage standards must be clearly explained. When establishing the standards, such information as the estimated daily intake (see Chapter II, Article 4) and the ADI obtained from toxicological tests should be taken into consideration.
   (2) When the establishment of usage standards is determined to be unnecessary, the reasons for such determination should be clearly indicated.
7. Other (Information useful for assessments of the effect of food on health)

Article 2. Findings regarding safety

1. Disposition studies

Studies to examine the disposition within the body should comply with the disposition study guideline published by the Ministry of Health and Welfare in 1996. They also should follow the notes below.

(1) The food additive or substance labeled by an isotope should be used as the test substance. When an isotope-labeled substance is used, the species and location of the isotope should be clearly indicated.

(2) It is preferable to conduct tests on more than two species (more than one rodent species [typically rats] and more than one non-rodent species [typically 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 and other tests may be carried out when necessary in order to calculate accurate ratio of absorption or for other purposes.

(4) Each process of absorption, distribution, metabolism, and excretion must be examined and values recorded, such as concentration of the active ingredient in the blood; amount of the substance in urine, feces and other excretory matter; and successive changes in the concentration in each organ; metabolites found in organisms, as well as factors that are influential in each step.

(5) The results regarding absorption, distribution metabolism and excretion (e.g., highest concentration in blood plasma, successive change in concentration in each organ, and elimination half-life) should be used to determine the organ(s) that can be a target of toxicological tests. In such cases, the feasibility of extrapolating the results to obtain the effects on the human body must be examined with regard to differences among animal species and species specificity.

(6) For tests using a racemic body, it is preferable to examine the disposition of each optical isomer within the body if it is necessary to understand the association with toxicity.

(7) In principle, the existence of human-specific metabolites must be examined and toxicological tests of such metabolites must be carried out as necessary.

2. Toxicological studies

(1) Subchronic toxicity studies and chronic toxicity studies

(a) Tests should be conducted on one rodent species (generally rats) and one non-rodent species (generally dogs). In principle, the same number of male and female animals should be used.

(b) The administration period should be 28 days or 90 days for subchronic toxicology tests and more than 12 months for chronic toxicology tests. The 28-day test can be omitted when a test with a 90-day administration period is carried out.

(c) In principle, the test substance should be orally administered 7 days a week. The substance should be administered in animal feed or water, but it can be also administered by gavage.

(d) At least three groups receiving different levels of the administration dose should be established in addition to the control group. The reasons for choosing each dose level should be clearly indicated. Proper ratios should be chosen so that an appropriate NOAEL can be obtained.

(e) Care should be taken to prevent nutritional disturbance among test animals when

feeding them the substance. Usually, the amount of the substance as a proportion of the feed does not have to exceed 5% (W/W). When the substance is given by gavage administration, the general maximum dose needed is the technically possible maximum dose or 1,000 mg/kg bw. If no effect is observed at that dose, the administration of a higher dose is not required.

(f) When the frequency or severity level of a naturally occurring pathological change that is also observed within the control groups increases due to the administration of the substance, even within the context of the background data it should, in principle, be taken as an effect caused by the administration of the substance if biological some significance, such as a relationship between the dose and the frequency or severity level, is recognized.

(g) When neurotoxicity or immunotoxicity[3] is suspected, the need for additional tests as described in the OECD test guideline or ICH (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use) guideline should be examined.

(h) The procedure to extrapolate the findings of toxicological tests to humans should be examined carefully by analyzing the endpoints separately and for different factors, such as functional changes, non-oncological morphological changes, oncological morphological changes, and changes to reproductive functions.

(i) When a combination test for chronic toxicity and carcinogenicity is carried out using one rodent species, a chronic toxicity test and carcinogenicity test on another rodent species can be omitted. .

(j) The need to add an *in utero* exposure phase should be examined where necessary.

(2) Carcinogenicity studies

(a) Tests should be conducted on more than two rodent species (rats, mice or hamsters are used generally). In principle, the same number of male and female animals should be used.

(b) In principle, administration should be carried out orally 7 days a week. For rats, the period should be between 24 months or longer and 30 months or shorter. For mice, the period should be between 18 months or longer and 24 months or shorter. The test substance should be orally administered in animal feed or with water, but it can be also administered by gavage if oral administration is difficult.

(c) At least three groups receiving different levels of the administration dose should

[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.

be established in addition to the control group. The reasons for choosing each dose level should be clearly indicated. Proper ratios should be chosen so that an appropriate NOAEL can be obtained.

(d) Care should be taken to prevent nutritional disturbance among test animals when feeding them the substance. Usually, the amount of the substance as a proportion of the feed does not have to exceed 5% (W/W). When the substance is given by gavage administration, the general maximum dose needed is the technically possible maximum dose or 1,000 mg/kg bw. If no effect is observed at that dose, the administration of a higher dose is not required.

(e) If the test for carcinogenicity is positive, the ADI cannot be established in principle if genotoxicity is positive and the substance is determined to be a genotoxic carcinogen. If the test for carcinogenicity is negative, the ADI can be established if genotoxicity is negative and the substance is determined not to be a genotoxic carcinogen. Even if the food additive being assessed unavoidably generates/contains a byproduct/residue that is suspected of being genotoxic, the ADI may be established in some cases after a required examination (see Chapter I, Article 4-3, 4-4).

(f) If the incidence rate of lesions is relatively low, carcinogenicity may be determined during the assessment by conducting a significance test 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. Assessment of carcinogenicity, including precancerous lesions, is especially preferable where there is an increase in endocrine system tumors, a type of lesion that frequently occurs with rodent species.

(g) If an increase in tumors in a region where tumor incidence is not normally high or when an increase in rare tumors is recognized it is preferable to include the carcinogenic mechanism in the assessment.

(h) Factors that modify the development of cancer (suppression of weight increase or decrease of survival rate) should be taken into consideration for the assessment.

(i) Special attention should be paid to species-specific toxicological findings (e.g., hypertrophy, hyperplasia and tumor of thyroid follicle ~~epithelium [~~epithelium [specific to rodents] and renal disorder and tumor [specific to male rats]).

(j) When a combination test for chronic toxicity and carcinogenicity is carried out using one rodent species, a chronic toxicity test and carcinogenicity test on another rodent species can be omitted.

(k) The need to add an *in utero* exposure phase should be examined where

necessary.

(3) Toxicity/carcinogenicity combination studies with one-year repeated-dose administration

Notes in (1) and (2) should be followed.

(4) Reproductive toxicity studies

Studies to examine reproductive toxicity should comply with the reproductive toxicity study guideline published by the Ministry of Health and Welfare in 1996. They also should follow the notes below.

(a) Tests should be conducted on more than one rodent species (rats are used generally). In principle, the same number of male and female animals should be used.

(b) In principle, administration should be carried out orally 7 days a week. The test substance should be orally administered in animal feed or with water, but it can be also administered by gavage if oral administration is difficult.

(c) At least three groups receiving different levels of the administration dose should be established in addition to the control group. The reasons for choosing each dose level should be clearly indicated. Proper ratios should be chosen so that an appropriate NOAEL can be obtained.

(d) Care should be taken to prevent nutritional disturbance among test animals when feeding them the substance. Usually, the amount of the substance as a proportion of the feed does not have to exceed 5% (W/W). When the substance is given by gavage administration, the general maximum dose needed is the technically possible maximum dose or 1,000 mg/kg bw. If no effect is observed at that dose, the administration of a higher dose is not required.

(e) When neurotoxicity or immunotoxicity is suspected, the need for additional tests as described in the OECD test guideline or ICH guideline should be examined.

(5) Prenatal developmental toxicity studies

Studies to examine prenatal developmental toxicity should comply with the teratogenetic study guideline published by the Ministry of Health and Welfare in 1996 and the notes below. The minimum period of administration should be from the date of implantation to the estimated delivery date, and the substance should be administered daily to the pregnant animals.

(a) Tests should be conducted on more than two species (more than one rodent species [typically rats] and more than one non-rodent species [typically rabbits]).

(b) The test substance should be orally administered by gavage.

(c) At least three groups receiving different levels of the administration dose should be established in addition to the control group. The reasons for choosing each dose level should be clearly indicated. Proper ratios should be chosen so that an appropriate NOAEL can be obtained.

(6) Genotoxicity studies

Studies to examine genotoxicity should comply with the mutagenicity test guideline published by the Ministry of Health and Welfare in 1996. But the examination should not be limited to the narrow definition of "mutagenicity" and the assessment should be carried out based on the test results regarding genotoxicity in general. Among the tests included in the standard combination (i.e., combination of bacterial reverse mutation tests, chromosome aberration tests using cultured cells of mammals, and micronucleus tests on rodents), the chromosome aberration tests using mammalian cultured cells can be replaced with a mouse lymphoma TK assay (MLA) or *in vitro* micronucleus test. In order to supplement the results from the standard test combination, single cell gel electrophoresis ("Comet Assay") and *in vivo* transgenic animal mutation assay can be used, in addition to those described in the Ministry of Health and Welfare guideline of 1996.

If one of the tests in the standard combination cannot be conducted due to technical constraints, the reason should be explained backed up by scientific evidence. One of the internationally validated tests can be used as a replacement.

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

(a) If the results of the bacterial reverse mutation tests are positive, a comprehensive judgment should be made by fully considering the results of *in vivo* tests that use genetic mutation or DNA damage (Comet Assay, *in vivo* transgenic animal mutation assay) as an indicator.

(b) If the results of the chromosome aberration tests using mammalian cultured cells are positive and the effect is also confirmed with rodent micronucleus tests, the substance can be determined as positive for genotoxicity.

(c) Even if the results of the chromosome aberration tests using mammalian cultured cells are positive, if the results of the rodent micronucleus tests (preferably with evidence to show exposure of the target organ) are negative, the substance can be determined as negative for genotoxicity.

(7) Allergenic potential studies[4]

Studies to examine the allergenicity of food additives should follow the antigenicity tests guideline published by the Ministry of Health and Welfare in 1996. There is no well-established method for predicting the allergenicity of chemical substances when orally ingested, particularly for predicting the immediate type of allergenicity. Therefore, studies should be carried out with sensitization and induction methods approved by specialists. For the time being, allergenicity studies using delayed allergy as an indicator should at least be carried out. 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 should follow the "Standards for the Safety Assessment of Genetically Modified Foods (Microorganisms)" (FSCJ decision, June 26, 2008).

(8) General pharmacological studies

Studies to examine general pharmacological properties of food additives should follow the general pharmacological test guideline published by the Ministry of Health and Welfare in 1996.

(9) Other studies

When neurotoxicity is suspected following a subchronic toxicity test and other tests, additional tests should be conducted as necessary in compliance with the OECD test guideline and other materials.

When immunotoxicity is suspected following a subchronic toxicity test and other tests, proper immunofunctional tests should be added as necessary in accordance with the ICH guideline and other materials. Immunofunctional tests should be also carried out as necessary when immunotoxicity in humans is suspected based on existing findings.

Article 3. Findings in humans

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

[4] Also referred to as "allergenicity"

Article 4. Estimation of daily intake

1. The daily intake should be determined based on the Japanese diet. Care should be taken to avoid intake estimations that are too small. In principle, the estimated daily intake is calculated by multiplying the daily intake of the food items for which the additive is to be used by the amount of additive used. The daily intake of food should be properly estimated based on the food group intakes given in the National Health and Nutrition Survey or other materials. Estimations based on data gathered using other reliable methods, such as market basket surveys and production analysis, can also be used. The daily intake should be estimated for body weight of 50 kg.
2. The estimated daily intake should be compared with the ADI obtained from toxicological tests, and the results of such comparison should be examined. Where necessary, the safety of food additives should also be examined in cases where more than one item of the same kind of food additive, etc. is simultaneously consumed. This can be done by comparing the sum of estimated daily intake to the group ADI, or by any other method.
3. Where considered necessary based on food consumption habits in Japan, the overconsumption of nutritional elements and effects on electrolyte balance should also be examined along with other relevant effects.

Article 5. Assessment procedure for "Internationally commonly used flavors

Assessments of internationally commonly used flavors should be conducted based on the Safety Assessment Procedures regarding internationally commonly used Flavors (last report, second revision) (November 4, 2003). Although this material indicates the need for *in vivo* tests in cases where genotoxicity in living organisms is suspected following an *in vivo* genotoxicity test using microorganisms and mammalian cells, additional *in vivo* chromosome aberration tests are not needed if the results of an *in vivo* micronucleus test are already available.

For the estimation of intake, the JECFA is planning to adopt the single portion exposure technique (SPET) method, a method for estimating the total intake by estimating the proportional amount of additives contained in foods in each food group, in addition to the conventionally used per capita intake times ten (PCTT) method. The results of the SPET method will be taken into account in future assessments. For Japan, where the estimation of the proportional amount of food additives is not practical for new additives, the PCTT method will continue to be used as the assessment method and the adaptation of the SPET method will be discussed as an issue.

Article 6. Assessment methods for enzymes

Safety assessments of enzymes are, in principle, carried out based on the data in Appendix 1 and other information. When the safety of a production strain is not known for enzymes obtained from microorganisms, appropriate tests must be conducted to assess the safety of the original microorganism. Pathogenic or toxin-producing production bacteria should not in principle be used for the production of enzymes.

When it is scientifically proven that the enzyme is broken down in the digestive tract to become a common component of food*, the materials regarding toxicity listed in Appendix 1 can be omitted. The materials regarding toxicity listed in Appendix 2 should be submitted.

* Such judgment should be made by considering the items in Table 2 in the guideline published by the Ministry of Health and Welfare in 1996

Article 7. Assessment methods for nutritional elements

For the safety assessment of nutritional elements that are biologically essential or nutritional elements that are proven to have positive effects on human health when consumed at a certain level, materials listed in Appendix 1 are required, in principle. 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) should be also referred to for assessment.

Assessments of nutritional elements should be conducted by considering the following notes.

1. Assessment should be conducted comprehensively based on the findings in humans from clinical tests, epidemiological studies, and case reports. In assessments, background factors and variation in quality of studies should be taken into consideration and the findings obtained from meta-analysis should be weighted higher.
2. The range of the amounts required and the amounts consumed in humans are frequently relatively close to the LOAELs or NOAELs reported for humans. This fact, as well as the homeostatic function specific to the nutritional element, should be taken into consideration in the adoption of uncertainty factors[5] that differ depending on the nutritional element.
3. When an excessive intake amount is likely to have serious effects on human health and in other cases where the amount of habitual intake from food is taken into consideration as background, the distribution of habitual intake, in addition to the average value, should be examined where necessary.

[5] The safety factor used for establishing a tolerable upper limit for nutritional elements in food intake standards.

4. When a tolerable upper limit of the nutritional element is shown in the Dietary Reference Intakes for Japanese established by the Ministry of Health, Labour and Welfare, the figures shown and relevant background data should be examined.

Appendix 1. Materials required for assessments of food additives (excluding "Internationally commonly used flavors)

Items		Designation	Revision of standard
Information on the additive subject to assessment			
	1. Name and usage	Required	Required
	2. Origin or process of discovery	Required	*
	3. Usage in other countries	Required	Required
	4. Assessments by international organizations and other organizations	Required	*
	5. Physiochemical properties	Required	*
	6. Suggestions for usage standards	Required	Required
	7. Others (Information useful for assessments of the effect of food on health)	*	*
Findings regarding safety			
	1. Tests for disposition in organisms	Required	*
	2. Toxicity		
	(1) Subchronic toxicity studies and chronic toxicity studies	Required	*
	(2) Carcinogenicity studies	Required	*
	(3) Toxicity/carcinogenicity combination studies with one-year repeated-dose administration	Required	*
	(4) Reproductive toxicity studies	Required	*
	(5) Prenatal developmental toxicity studies	Required	*
	(6) Genotoxicity studies	Required	*
	(7) Allergenic potential studies	Required	*
	(8) General pharmacological studies	Required	*
	(9) Other studies	*	*
	3. Findings in humans	Required	*
	4. Estimation of daily intake, etc.	Required	Required

Note 1. When requesting a division of usage standards for a food additive for which assessment of the effect of the food on health has already been carried out by FSCJ, the materials required for "Revision of standard" should be submitted. When requesting a division of usage standards for a food additive for which assessment of the effect of the food on health has not been carried out by FSCJ, documents required for designation should be submitted, in principle.

Note 2. Materials marked "Required" should be submitted whenever applicable. Materials marked with an asterisk (*) should be submitted as necessary (when there is a new finding, for example).

Note 3. When a combination test for chronic toxicity and carcinogenicity is carried out using one rodent species, a chronic toxicity test and carcinogenicity test on another rodent species can be omitted.

Appendix 2. Materials regarding toxicity required for assessments of enzymes (when it is scientifically proven [by considering the items in Table 2 in the guideline published by the Ministry of Health and Welfare in 1996] that the enzyme is broken down in the digestive tract to become a common component of food)

Item	Designation	Revision of standard
(1) Toxicity tests on rats with 90-day repeated administration	Required	*
(2) Genotoxicity tests	Required	*
(3) Allergenicity tests	Required	*

Note: Until further notice, the tests necessary for allergenicity assessment should be determined by referring to the “Standards for the Safety Assessment of Genetically Modified Foods (Microorganisms)” (FSCJ decision, June 26, 2008).