Category number	Foods	Restrictions/exception	Maximum use level proposed for advantame (mg/l or mg/kg as appropriate)
1.4	Flavoured fermented milk products including heat-treated products	only energy-reduced products or with no added sugar	20
3	Edible ices	only energy-reduced or with no added sugar	16
4.2.2	Fruit and vegetables in vinegar, oil, or brine	only sweet-sour preserves of fruit and vegetables	6
4.2.3	Canned or bottled fruit and vegetables	only fruit energy-reduced or with no added sugar	20
4.2.4.1	Fruit and vegetable preparations excluding compote	only energy-reduced	20
4.2.5.1	Extra jam and extra jelly as defined by Directive 2001/113/EEC	only energy-reduced jams jellies and marmalades	20
4.2.5.2	Jam, jellies and marmalades and sweetened chestnut puree as defined by Directive 2001/113/EEC	only energy-reduced jams, jellies and marmalades	20
4.2.5.3	Other similar fruit or vegetable spreads	only dried-fruit-based sandwich spreads, energy-reduced or with no added sugar	20
5.1	Cocoa and Chocolate products as covered by Directive 2000/36/EC	only energy-reduced or with no added sugars	40
5.2	Other confectionery including breath refreshing microsweets	only cocoa or dried fruit based, energy reduced or with no added sugar	40
5.2	Other confectionery including breath refreshing microsweets	only cocoa, milk, dried fruit or fat based sandwich spreads, energy-reduced or with no added sugar	20
5.2	Other confectionery including breath refreshing microsweets	only starch based confectionary energy reduced or with no added sugar	40
5.2	Other confectionery including breath refreshing microsweets	only confectionary with no added sugar	20
5.2	Other confectionery including breath refreshing microsweets	only breath-freshening micro-sweets, with no added sugar	120
5.2	Other confectionery including breath refreshing microsweets	only strongly flavoured freshening throat pastilles with no added sugar	40
5.3	Chewing gum	only with added sugars or polyols, as flavour enhancer	200
5.3	Chewing gum	only with no added sugar	400

. Findings regarding safety

iii. Findings regarding safety

1. Disposition studies

Disposition studies are intended to obtain information on the pharmacokinetics (absorption, distribution, metabolism, and excretion) of a test substance after its administration in animals in order to estimate the pharmacokinetics and development of adverse effects in humans. Discussions that contribute to the evaluation of toxicity studies or their results should also be included whenever possible.

The following are noted in the assessment guidelines by the FSCJ.

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.

This study will include tests conducted in accordance with the guideline published by the Ministry of Health and Welfare in 1996. However, other appropriate methods may be considered depending on the nature of the test substance, and tests based on the OECD test guidelines or ICH (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use) guidelines, for example, may be selected or other tests may be substituted as befits the purpose of the study. Appropriate data on the pharmacokinetics of the test substance that has been obtained from toxicity studies may also be used.

Notes

- When existing evaluation reports are cited, the study source should be identified.
- The species, strains, gender, and number of test animals, as well as the method of administration, vehicle, dose, and method of labeling should be clearly indicated.
- Results should be tabulated for ease of comprehension, but information that is not amenable to tabulation should be described in detail in individual paragraphs.
- When residue levels are evaluated from the test that uses radioisotope, it is preferable to be described as residual radiation level (%TRR or %TAR) or residual concentration (mg/kg or μg/kg).
- Assessments such as of pharmacokinetics and the development of adverse effects in humans should also be discussed whenever possible.

*When related to a common component of food ("when scientifically known to be a common component of food or to be broken down in food or in the digestive tract into a common component of food"), test results showing the validity of the following based on items in Table 2 of the guideline published by the Ministry of Health and Welfare in 1996 should be noted.

(1) Relevance to a common component of food

① Under conditions in which food additives are commonly used, the substance must be readily broken down in food or in the digestive tract into a substance that is identical with a common component of food.

② Major factors involved in the breakdown of the substance in food or in the digestive tract (such as pH or enzymes) must be ascertained.

③ When the proper amount is used under conditions in which food additives are commonly used, the food additive must be absorbed in the body to the same extent as food components and must not interfere with the absorption of other nutritional components.

④ Non-hydrolysates or partial hydrolysates of the ingested food additive cannot be excreted in large amounts in feces. Non-hydrolysates or partial hydrolysates also cannot accumulate in biological tissue.

⑤ Ingestion of food in which the food additive is used cannot result in excessive ingestion of the primary component of the food.

*When not relevant to a common component of food, test results for the following should be noted for each animal species.

(1) Absorption

a. Blood concentration-time profile

Information such as the maximum concentration in blood (Cmax) after dosing, the time to reach the maximum concentration (Tmax), and the area under the blood concentration-time curve (AUC) should be noted for each test animal to show the extent and rate of test substance absorption.

It is also useful to discuss comparisons of these parameters with the same parameters after intravenous administrations or other standard administration methods.

b. Absorption rate

The level of urinary, fecal, biliary, and respiratory excretion, for example, after administration of the test substance as well as the absorption rate in the body calculated on the basis of the above total excretion level should be described.

(2) Distribution

The organ and tissue distribution, as well as the changes and accumulation over time after single and repeated doses of the test substance, should be described for each test animal. The results of measurements at several time points should preferably be described in order to accurately reflect the pharmacokinetics.

Organs and tissues characterized by high concentrations of distribution or accumulation and by adverse reactions as a result of repeated doses should preferably be discussed, as should their form.

(3) Metabolism

To provide information on the metabolic pathway and the extent and rate of metabolism, quantitative values for unchanged compound and metabolites in biological samples, such as blood, urine, bile, and feces, after single and repeated doses should be described for each test animal.

In vitro tests of samples of the organs involved in metabolism, such as slices, homogenates, cell suspensions, and cell fractions, may also be described.

(4) Excretion

The levels of urinary, fecal, respiratory, biliary, lactic or other excretion over time after single and repeated doses should be described for each test animal to provide information on the excretory pathway of the test substance and principal metabolites, as well as the extent and rate of their excretion.

(Examples of descriptions)

When writing up the descriptions, existing evaluation reports at https://www.fsc.go.jp/fsciis/evaluationDocument/list?itemCategory=000 can be used as reference. The following are typical examples.

(1) Absorption

① Absorption in rats

a. Blood concentration profile

According to the report by XX [name of author] (XX [year of report]), a GLP-compliant study was conducted to analyze blood concentration profiles after the XX administration [method of administration] of XX [test substance] (XX, XX, XX mg/kg bodyweight/day) for XX [time per period] in XX-old XX [animal species] (X males and

females each per group [group establishment]). As shown in Table 1, the results showed that the blood concentrations of XX [test substance] in the XX dose group(s) peaked (XX to XX mg/L) at X hours post-dose, and was XX at X hours post-dose and XX at X hours post-dose, with a $T_{1/2}$ of X hours and an AUC of X μg•hr/g (Ref. X).

Table 1: Pharmacokinetics parameters in blood

Gender	Dose (mg/kg body weight)	Tmax (hr)	Cmax (μg/g)	$T_{1/2}$ (hr)	AUC (μg・hr/g)

b. Absorption rate

According to the report by XX [name of author] (XX [year of report]), a GLP-compliant study was conducted to analyze the *in vivo* absorption rate after the XX administration [method of administration] of XX [test substance] (XX, XX, XX mg/kg bodyweight/day) for XX [time per period] in XX-old XX [animal species] (X males and females each per group [group establishment]). Based on the radioactive concentration in test samples [such as urine, cage wash, feces, and bile], the *in vivo* absorption rate in the X-dose group(s) was estimated to be at least X% (Ref. X).

(2) Distribution

① Distribution in rats

According to the report by XX [name of author] (XX [year of report]), a GLP-compliant study was conducted to analyze *in vivo* distribution after the XX administration [method of administration] of XX [labeled test substance] (XX, XX, XX mg/kg bodyweight/day) for XX [time per period] in XX-old XX [animal species] (X males and females each per group [group establishment]). As shown in Table 2, the results revealed that XX [test substance] was distributed in high concentrations in XX and XX at X hours post-dose, but that the distribution peaked in XX at X hours post-dose and was XX at X hours post-dose (Ref. X).

Table 2: Total radioactivity level in tissues after XX administration of X-labeled XX in rats (%TRR, etc.)

Tissue	Time after dose (hours)			
Liver				
Kidney				
Large intestine				
Muscle				
Plasma				

Whole blood				
Milk				

(3) Metabolism

① Metabolism in rats

According to the report by XX [name of author] (XX [year of report]), a GLP-compliant study was conducted to identify metabolites in XX and XX after the XX administration [method of administration] of XX [labeled test substance] (XX, XX, XX mg/kg bodyweight/day) for XX [time per period] in XX-old XX [animal species] (X males and females each per group [group establishment]). As shown in Table 3, XX and XX were found as the unchanged compound and metabolite of XX [test substance] (Ref. X).

Table 3: Radioactivity level of XX and metabolites after XX administration of X-labeled XX in rats (%TRR, etc.)

Number of doses	Dose (mg/kg body weight)	Gender	Samples	Unchanged compound	Metabolites (%TRR)
Single dose			Blood		A ( ), B ( ), C ( ), D ( ), and sulfate conjugate of D ( )
			Urine		
			Bile		
			Feces		
Repeated doses					

(4) Excretion

① Excretion in rats

According to the report by XX [name of author] (XX [year of report]), a GLP-compliant study was conducted to analyze over time the excretion rate in urine and feces after the XX administration [method of administration] of XX [labeled test substance] (XX, XX, XX mg/kg bodyweight/day) for XX [time per period] in XX-old XX [animal species] (X males and females each per group [group establishment]). As shown in Table 4, the results showed that X% of XX [test substance] was excreted in XX at X hours post-dose, and X% was excreted in XX at X hours post-dose. The principal excretory pathway was XX (Ref. X).

Table 4: Percent excreted (%TRR) in urine and feces at X and X hours-post dosing

Number of doses	Dose (mg/kg body weight)	Gender	Samples	X hours post-dosing	X hours post-dosing	Total
			Urine			

			Feces			

2. Toxicological studies

Toxicological studies are intended to obtain information on the effects of the administration of a test substance in animals in order to deduce, for example, the ways in which adverse effects develop in humans and the doses at which they occur.

The following are noted in the assessment guidelines by the FSCJ.

(1) Subchronic toxicity studies and chronic toxicity studies

① 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.

② 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.

③ 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.

④ 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.

⑤ 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.

⑥ 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.

⑦ When neurotoxicity or immunotoxicity[*1] 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.

⑧ 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.

⑨ 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.

⑩ The need to add an *in utero* exposure phase should be examined where necessary.

[*1] 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.

(2) Carcinogenicity studies

① 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.

② 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.

③ 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.

④ 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.

⑤ 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.

⑥ 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.

⑦ 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.

⑧ Factors that modify the development of cancer (suppression of weight increase or decrease of survival rate) should be taken into consideration for the assessment.

⑨ Special attention should be paid to species-specific toxicological findings (e.g., hypertrophy, hyperplasia and tumor of thyroid follicle epithelium [specific to rodents] and renal disorder and tumor [specific to male rats]).

⑩ 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.

⑪ 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.

① 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.

② 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.

③ 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.

④ 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.

⑤ 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.

① 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]).

② The test substance should be orally administered by gavage.

③ 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.

① 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.

② 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.

③ 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[*2]

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

[*2] Also referred to as "allergenicity"

tests should be also carried out as necessary when immunotoxicity in humans is suspected based on existing findings.

This study will include tests conducted in accordance with the guideline published by the Ministry of Health and Welfare in 1996. However, other appropriate methods may be considered depending on the nature of the test substance, and tests based on the OECD guidelines or the ICH guidelines, for example, may be selected or other tests may be substituted as befits the purpose of the study.
Acute toxicity study information may also be included.
If a 90-day repeated-dose toxicity study is conducted, there will be no need to conduct a separate 28-day repeated-dose toxicity study in the same species.
If one-year repeated-dose toxicity and carcinogenicity studies are conducted in the required species, there will be no need to conduct a combined one-year repeated-dose toxicity/carcinogenicity study. Conversely, if a combined one-year repeated-dose toxicity/carcinogenicity study is conducted in a rodent species, there will be no need to conduct separate one-year repeated-dose toxicity and carcinogenicity studies in a rodent species.

*When the additive being assessed is related to a common component of food ("when scientifically known to be a common component of food or to be broken down in food or in the digestive tract into a common component of food"), there will be no need to attach toxicity-related data, as per the guideline published by the Ministry of Health and Welfare in 1996, but materials on 28-day repeated-dose toxicity studies in rodents and genotoxicity studies should preferably be attached.

*Regarding assessment methods for enzymes, FSCJ says "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 (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), the materials regarding toxicity listed in Appendix 1 can be omitted. The materials regarding toxicity listed in Appendix 2[*1] should be submitted."

[*1] Materials listed in Appendix 2: materials on (1) 90-day repeat-dose toxicity studies in rodents, (2) genotoxicity studies and (3) allergenicity studies

Notes

- When existing evaluation reports are cited, the study source should be identified.
- In principle, tests involving oral administration should be described.
- Results should preferably be tabulated for ease of comprehension, but information that is not amenable to tabulation should be described in detail in individual paragraphs.
- The bacterial strains, types of cells, the animal species, strains, gender, and number of test animals, the method of administration, vehicle, and dose should be clearly indicated.
- Food additive degradation products and contaminants should also be studied as needed.
- The doses used in tests should preferably be indicated in units of "mg/kg body weight per day."
- The GLP status of the tests should preferably be indicated.

1) Acute toxicity studies

- The results of acute toxicity studies should preferably be expressed as the LD_{50} (median lethal dose), for example.

2) Subchronic toxicity studies

- The results of typical repeated-dose toxicity studies (28-day and 90-day repeated-dose toxicity studies) should be described in this section.
- Information on toxicity findings and the doses at which they occurred (with statistical analysis) should be described.
- Information on the NOAEL or LOAEL should be described.

3) Chronic toxicity studies and carcinogenicity studies

- The results of typical life-long, chronic repeated-dose toxicity studies (one-year repeated-dose toxicity studies and carcinogenicity studies, and combined one-year repeated-dose toxicity/carcinogenicity studies) should be described in this section.
- The format should be based on that of "subchronic toxicity studies," but the main point of carcinogenicity studies is whether or not the additive is carcinogenic.

4) Reproductive toxicity studies

- Information on the reproductive functions of males and females, estrus cycle, mating behavior, conception, delivery, lactation, and development and behavior of offspring in reproductive testing (multigeneration reproductive toxicity studies) should be noted.
- Information related to the effects on fetal development in teratogenicity studies (prenatal development toxicity studies) should be noted.

5) Genotoxicity studies (mutagenicity test)

- The technical product and, if necessary, metabolites should be described under the separate categories of *in vitro* tests (such as microbial reverse mutation assay and chromosomal aberration assay in cultured mammalian cells) and *in vivo* tests (rodent micronucleus assay).
- It should be clearly indicated whether any metabolic activator was added or not.

6) Other studies

- Special studies such as allergenic potential studies, general pharmacology studies, neurotoxicity studies, and immune function studies should be described as needed.

(Examples of descriptions)

When writing up the descriptions, existing evaluation reports at https://www.fsc.go.jp/fsciis/evaluationDocument/list?itemCategory=000 can be used as reference. The following are typical examples.

The results of multiple acute toxicity and genotoxicity studies should preferably be tabulated collectively, but the results of repeated-dose toxicity studies, for example, should preferably be tabulated for each study. Information that is not amenable to tabulation may also be described in detail in individual paragraphs.

1) *In vivo* toxicity studies

Acute toxicity studies

The results of acute toxicity studies on XX [test substance] and its metabolites in rats and mice are presented in Table 5 (Ref. X).

Table 5: Summary of acute toxicity study results

Test substance	Route of administration	Species	LD_{50} (mg/kg body weight)		Observed symptoms	Reference
			Males	Females		
XX	Oral	SD rats	>5,000	>5,000	Watery stool	XX, Year
		ICR mice	>4,000	>4,000	1 death at 1,000 mg/kg body weight	XX, Year
	Percutaneous	F344 rats	2,500	3,000	No symptoms or deaths	XX, Year
	Inhalation	SD rats	>10	>10	Diarrhea, blepharoptosi	XX, Year

					s	
Metabolite A	Oral	SD rats	>2,000	>2,000	Watery stool	XX, Year

Repeated-dose toxicity studies/carcinogenicity studies

<1. In case of summarizing results using tables>

According to the report by XX [name of author] (XX [year of report]), a GLP-compliant study was conducted on the XX administration [method of administration] of XX [test substance] (XX, XX, XX mg/kg bodyweight/day) for XX [period] in XX-old XX [animal species] (X males and females each per group [group establishment]) setting administered group as Table 6 (Ref. X).

Table 6: Dosage level

Dosage level (% or ppm)	A, B, C
Equivalent to mg/kg body weight/day	A', B', C'

The results showed no treatment-related effects on observation parameters such as general condition, body weight, food consumption, water consumption, blood biochemistry, urinalysis, ophthalmology. Blood biochemistry revealed elevated ALT and AST levels in males and females of the B' mg/kg bodyweight/day and higher dose groups. Elevated sodium level was also observed in males of the B' mg/kg/body weight/day group. This was not considered to be toxic changes because no other related electrolyte changes or dose-response relationships were found.

Analysis of organ weight revealed increases in the absolute and relative weight of the liver in males of the B' mg/kg/ body weight/day and higher dose groups and in females of the C' mg/kg body weight/day dose group. Histopathology revealed centrilobular hepatocyte hypertrophy in males and females of the B' mg/kg body weight/day and higher dose groups, and single cell necrosis of hepatocytes in males of the C' mg/kg body weight/day dose group. These findings were determined to indicate toxicity because they were consistent changes characterized by a dose-response relationship. The NOAEL was, thus, assessed as A' mg/kg/ body weight/day in this study.

Table 7: Toxic findings in XX [study title] toxicity study (XX [animal species])

Dose	Males	Females
C' mg/kg body weight/day	Single cell necrosis of hepatocytes	Increases in absolute and relative weight of liver
≥ B' mg/kg body weight/day	Elevated ALT and AST Increases in absolute and relative	Elevated ALT and AST Centrilobular hepatocyte hypertrophy

	weight of liver Centrilobular hepatocyte hypertrophy	

<2. In case of summarizing results without tables>

According to the report by XX [name of author] (XX [year of report]), a GLP-compliant study was conducted on the XX administration [method of administration] of XX [test substance] (XX, XX, XX mg/kg bodyweight/day) for XX [period] in XX-old XX [animal species] (X males and females each per group [group establishment]) setting administered group. The results showed no treatment-related effects on XX [individually noted observation parameters such as general condition, body weight, food consumption, and water consumption, and test parameters such as hematology, blood biochemistry, urinalysis, ophthalmology or other functional tests, necropsy, or histopathology]. XX [findings] in XX [individually noted observation parameters such as general condition, body weight, food consumption, and water consumption, and test parameters such as hematology, blood biochemistry, urinalysis, ophthalmology or other functional tests, necropsy, or histopathology] were noted in XX [males and females] in the XX [dose] group. These findings were (or were not) determined to indicate toxicity based on XX [reasons]. The NOAEL (LOAEL) was, thus, assessed as XX [dose] in this study. (Ref. X)

The style is essentially the same as in Reproductive toxicity studies.

Observation and test parameters in reproductive testing include general condition, body weight, food consumption, water consumption, parameters related to pregnancy and delivery (such as copulation rate, pregnancy rate, and birth rate), neonatal parameters (such as number of pups, number of dead pups, number of live pups, external anomalies, and results of necropsy), results of necropsy, and histopathology.

Observation and test parameters in teratogenicity studies include general condition, body weight, food consumption, water consumption, and necropsy results for dams and fetuses.

According to the report by XX [name of author] (XX [year of report]), a GLP-compliant, two-generation reproductive study was conducted on the administration of XX [test substance] mixed with feed (A, B, and C ppm) for in XX-old XX [animal species] (X males and females each per group [group establishment]). The P generation parent animals mated and produced pups twice (offspring animals: F_{1a}, F_{1b}), F_{1b} animals were used as the F_1 generation parent animals, and they mated and produced pups twice (offspring animals: F_{2a}, F_{2b}). Analysis of the parent animals revealed suppressed weight gain in P generation males and females and in F_1 generation females in the C ppm dose group. Food consumption was also lower during the entire study period in P generation females and during the lactation periods of the two F_1 generations. Analysis of the offspring revealed lower 4-day postnatal survival rates in both F_1 and F_2 offspring as well as suppressed weight gain in F_{1b}, F_{2a}, and F_{2b} offspring in the C ppm dose group. These findings appeared to be secondary to the toxic effect of the test substance in parent animals. The NOAEL in this study was thus B ppm for parent and offspring animals (P males: b mg/kg body weight/day; P females: e mg/kg body weight/day; F_1 males: h mg/kg body weight/day; F_1 females: k mg/kg body weight/ day), with no findings of teratogenicity (Ref. X).

Table 8: Mean test article consumption in 2-generation reproductive study (XX [animal species])

Dose group			A ppm	B ppm	C ppm
Mean food consumption (mg/kg body weight/day)	P generation	Males	a	b	c
		Females	d	e	f
	F_1 generation	Males	g	h	i
		Females	j	k	l

Table 9: Toxic findings in 2-generation reproductive study (XX [animal species])

	Dose	1st generation (parents: P; offspring: $F_{1a,1b}$)		2nd generation (parents: F_{1b}; offspring: $F_{2a,2b}$)	
		Males	Females	Males	Females
Parent animals	C ppm	Suppressed weight gain	Suppressed weight gain Decreased food consumption	No toxic findings	Suppressed weight gain Decreased food consumption
Offspring animals	C ppm	Lower survival rate in nursing pups Suppressed weight gain	Lower survival rate in nursing pups Suppressed weight gain	Lower survival rate in nursing pups Suppressed weight gain	Lower survival rate in nursing pups Suppressed weight gain

2) Genotoxicity studies (mutagenicity test)

Table 10: Summary of *in vitro* genotoxicity studies

Type of test	Test subject	Test substance	Treatment concentration and dose	Results	Reference
Reverse mutation assay	*S. typhimurium* (TA XX, TA XX strain)		X to X mg/plate (+/-S9)	Negative	XX, Year
	S. typhimurium (TA XX, TA XX strain)		X to X mg/plate (+/-S9)	Positive	XX, Year
Chromosomal aberration	Chinese hamster ovary cells (CHO cells)		X to X mg/mL (-S9) X to X mg/mL (+S9)	Negative	XX, Year

assay	Human peripheral blood lymphocytes		X to X mg/mL (-S9) X to X mg/mL (+S9)	Negative Positive	XX, Year

Table 11: Summary of *in vivo* genotoxicity studies

Type of test	Test subject	Test substance	Treatment concentration and dose	Results	Reference
Micronucleus assay	XX mice; 5 males and females each (bone marrow cells)		X, X, and X mg/kg body weight (single oral dose)	Negative	XX, Year
	XX mice; 5 males and females each (hepatocytes)		X, X, and X mg/kg body weight (single oral dose)	Positive	XX, Year
Reporter gene transgenic animal mutagenicity assay	*gpt* delta mice; 5 males and females each (liver, kidneys)		X, X, and X mg/kg body weight (X-week oral dosing)	Negative	XX, Year

3. Findings in Humans

Because the purpose is to deduce safety and adverse effects in humans, the available information in humans should be noted.

The following are noted in the assessment guidelines by the FSCJ.

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.

Studies in humans include epidemiological studies, clinical experience, observations in case studies, research of the effects on health in humans during occupational exposure, reports of poisoning, and allergy studies in volunteers.

Notes

- When existing evaluation reports are cited, the study source should be identified.
- Gender, age, number of individuals, health status, and dosing method and dose should be noted.

(Examples of descriptions)

Intervention studies

According to the report by XX [name of author] (XX [year of report]), a randomized clinical study was conducted in XX Year in XX [location], in which XX- to XX-year old (average age XX) XX [study population] were randomized by a double-blind method to a placebo group (XX subjects) or an XX [test substance] (X mg/kg body weight/day) ingestion group for oral ingestion XX times a day [dosing method (such as capsules at breakfast)] for XX [period]. The results revealed no test substance treatment-related effects on XX [observation parameters such as general condition, hematology, blood biochemistry, urinalysis] (revealed that XX was affected). (Ref. X)

Cohort studies

According to the report by XX [name of author] (XX [year of report]) cited in the report of XX [assessment document source], an XX-year cohort study was conducted in X [gender] XX subjects (XX to XX years of age) in XX [location]. XX patients contracted XX [disease]. The relative risk for XX [disease] was XX (95% CI = XX to XX) in the X mg/kg body weight/day and higher dose groups when compared to groups with XX [test substance] consumption < X mg/kg bodyweight/day, revealing that XX [test substance] consumption ≥ X mg/kg body weight/day was strongly correlated to increased risk for XX [disease]. (Ref. X)

Other studies

No reports on studies of the oral administration of this test product in humans have been found, but the following related data is available from XX [name of author] (XX [year of report]).

When XX [test substance] (X mg/kg body weight/day) was orally administered for XX [period] to patients with XX, there were no medically abnormal findings in any subjects, and X% of the ingested amount was detected in urine. (Ref. X)

4. Estimation of Daily Intake

The Guideline for Assessment of the Effect of Food on Human Health Regarding Food Additives from the FSCJ describes as follows:

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 additives 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.

For the estimation of daily intake of food additives, (1) the calculation by multiplying the daily intake of the food items for which the additives to be used by the amount of additive used, (2) the market basket method, and (3) the method according to production amount survey of the additive are employed. These methods (1) through (3) are introduced below.

Notes

- If maximum usage concentrations are established in the use standards, applicants should estimate with the calculation by multiplying the daily intake of the food items for which the additives to be used by the amount of additive used, as a general rule.
- If subject food products are added through amendments to use standards, applicants should estimate not only the current intake, but also the increased intake after this addition.