Implementing rules of “Data Requirement for Supporting Registration of Agricultural Chemicals”

The Notification, Ref. No. 13-Seisan-3986, issued on 10 October, 2001 by the Director, Agricultural Production Materials Division, Agricultural Production Bureau, Ministry of Agriculture, Forestry and Fisheries of Japan

The Notification, Ref. No. 14-Seisan-7270, partly revised on December 10, 2002
The Notification, Ref. No. 16-Shoan-6198, partly revised on November 24, 2004
The Notification, Ref. No. 16-Shoan-9261, partly revised on March 16, 2005
The Notification, Ref. No. 18-Shoan-14852, partly revised on April 2, 2007
The Notification, Ref. No. 19-Shoan-14967, partly revised on March 31, 2008
The Notification, Ref. No. 21-Shoan-14387, partly revised on April 1, 2010
The Notification, Ref. No. 22-Shoan-10016, partly revised on April 1, 2011
The Notification, Ref. No. 25-Shoan-631, partly revised on May 31, 2013
The Notification, Ref. No.26-Shoan-533, partly revised on May 15, 2014

(Appendix)

Implementing rules of "Data Requirements for Supporting Registration of Agricultural Chemicals (The Notification, Ref. No. 12-Nousan-8147, issued on November 24, 2000 by the Director-General, Agricultural Production Bureau, Ministry of Agriculture, Forestry and Fisheries of Japan; hereinafter referred to as "the Notification by the Director-General")"

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Section 4 Regarding the Annex to the Notification by the Director-General "Guidelines on Preparation of Test Results Submitted When Applying for Registration of Agricultural Chemicals"

Supplementary Provisions (April 1, 2011)

1. The amendments in accordance with this notification shall apply to test results regarding efficacy, phytotoxicity, toxicity and persistence of agricultural chemicals that are submitted on and after April 1, 2011. However, the amendments defined in the following items shall apply to the tests or test results stipulated in each item.
(1) The amendments pertaining to item (3) of paragraph 7 of "3-1-1" of Section 5 of this "Implementing rules of "Data Requirement for Supporting Registration of Agricultural Chemicals"": The test initiated on and after October 1, 2011

(2) The amendments pertaining to paragraph (5) of Section 3 and item (2) of paragraph 7 of "3-1-1" of Section 5, and the amendments pertaining to Appendix Table 3-1, Appendix Table 3-2 and Appendix Table 6 of this "Implementing rules of "Data Requirement for Supporting Registration of Agricultural Chemicals"": The test results regarding residues in crops which are submitted on and after April 1, 2014 and fall under the following sub-item ① or ②:
① The test results pertaining to tests used for newly setting a standard value stipulated in "Specifications and Standards for Food, Food Additives, etc." (Ministerial Notification No. 370 of the Ministry of Health and Welfare, dated December 28, 1959) (referred to as "the standard value" in sub-item ②).
② The test results pertaining to tests used for changing the existing standard value

2. With regard to the test results which do not fall under sub-item ① nor ② of item (2) of the preceding paragraph, the provisions of the former notification remain applicable.

Supplementary Provisions (May 31, 2013)

The amendments in accordance with this notification shall apply to test results regarding efficacy, phytotoxicity, toxicity and persistence of agricultural chemicals that are submitted on and after May 31, 2013. However, with regard to the agricultural chemicals which are being registered at the time when this notification enters into force, the provisions of the former notification shall remain applicable. The amendments pertaining to "2-7-6" of Section 5 of this "Implementing rules of "Data

Requirement for Supporting Registration of Agricultural Chemicals"" shall apply to the tests initiated on and after December 1, 2013. The applicant may submit test results in accordance with the notification revised by this notification before December 1, 2013.

Supplementary Provisions (May 15, 2014)

1. The amendments in accordance with this notification shall apply to an application for registration of an agricultural chemical made on and after May 5, 2017. However, the amendments pertaining to "Section 1 Regarding test results to be submitted", "Annex Preparation of Summary of Test Results, etc." and Appendix Table 6 shall apply to the application for registration of an agricultural chemical made on and after May 15, 2014.

2. Notwithstanding the provisions of the main clause of paragraph 1, with regard to an application for registration of an agricultural chemical which is made by May 15, 2015 or an application for registration of an agricultural chemical which contains the same active ingredient as the agricultural chemical for which the test results had been submitted pursuant to the provisions of the notification prior to revision by this notification, the provisions then in force shall remain applicable.

3. The provisions of Section 4(7)② and Section 5 "Test on translocation to milk (3-1-2)" in the notification prior to revision by this notification(hereinafter, referred to as "the old notification") shall remain in force until May 15, 2017. In this case, with regard to the application of the provision of Section 4(7)②(ii), the phrase "shall refer to the cases where the residual level of the components, etc. of the relevant agricultural chemical in rice straw is 1 ppm or lower, or where the relevant agricultural chemical is not detected in forage crops on the basis of the test results regarding residues in crops" is deemed to be replaced with "shall refer to the cases where the relevant agricultural chemical is not detected in rice straw and forage crops on the basis of the test results regarding residues in crops.".

4. In the case referred to in the preceding paragraph, with regard to the provision of Section 5 "Test on translocation to milk (3-1-2)" 2 (3) in the old notification, the provision of Section 4 "Test on residues in livestock (3-1-2)" 5(2)② in Annex "Guidelines on Preparation of Test Results Submitted When Applying for Registration of Agricultural Chemicals" to the Notification by the Director-General revised on May 15, 2014 shall apply. However, in the case of tests that are commenced before May 15, 2014, this shall not apply.

Section 1 Regarding substitutes for test results

The details of substitutes for test results referred to in Section V, paragraph (2) of the Notification by the Director-general shall be specified as follows:

(1) Cases where an applicant applies for registration of an agricultural chemical which is identical to a registered agricultural chemical (limited to those for which 15 or more years have elapsed from the initial registration) in respect of the description of item (3) "Type of Agricultural Chemical" and items (4) to (6) of the Application Form for Agricultural Chemical Registration (Appended Form No.1 of the Enforcement Ordinance of the Agricultural Chemicals Regulation Law (1951 Ordinance of the Ministry of Agriculture and Forestry No.21)), and within the scope of applicable diseases or pests described in item (7) of that application form of the registered agricultural chemical shall fall under this case.

(2) The phrase "limited to test results listed in Section I, paragraph (3), items (a) to (c)" shall refer to test results regarding acute oral toxicity, acute dermal toxicity and acute inhalation toxicity using formulation as test substance.

(3) The phrase "limited to test results listed in Section I, paragraph (4), item (a)" shall refer to test results regarding residues in crops to be conducted for the other type of crops in cases where the tests have been conducted on 1 or more type of representative crop for each crop group (medium grouping).

Section 2 Regarding conditions necessary for conducting tests

(1) Type of test substance

The details of the "Type of test substance" column in the Appendix Table 1 of the Notification by the Director-General shall be specified as follows:

① Regarding formulation

Formulation used as test substance shall be equivalent to the sample of agricultural chemical to be submitted with an application for registration of agricultural chemical, in principle (hereinafter referred to as "the Sample"). In cases where the formulation used as test substance is not equivalent to the Sample, documents detailing the appropriateness of using the relevant formulation as test substance for each test item, including the one that shows that the difference between the formulation used as test substance and the Sample does not give any effect to the test results, shall be attached to the relevant test results.

In cases where treatment such as grinding and dissolution is applied to the formulation used as test substance during tests, sufficient attention shall be paid to that treatment not to give any effect to the test results.

As for spreader, its efficacy test, phytotoxicity test and test on residues in crops shall be conducted for each applicable crop in combination with the relevant agricultural chemicals to which the spreader is to be applied, in principle because a spreader is always used in combination with applicable agricultural chemicals.

② Regarding technical grade active ingredient (hereinafter referred to as "TGAI")

TGAI used as test substance shall be equivalent to the TGAI used as the raw material of the Sample, in principle.

In cases where the TGAI used as test substance is not equivalent to the TGAI used as the raw material of the Sample, documents detailing the appropriateness of using the relevant TGAI as test substance for each test item, including the one that shows that the difference between the TGAI used as test substance and the TGAI used as the raw material of the Sample does not give any effect to the test results, shall be attached to the relevant test results.

③ Regarding active ingredients, etc. that are either labeled or unlabeled with radioactive isotopes

(i) Test substances including the case of salt, etc. shall be identical to the active

ingredients in the formulation, in principle. However, even though substance different from the active ingredients is used as the test substance, if a scientific explanation can be made to prove that the results of the tests using relevant substance will not be different from the results of the tests using the very active ingredients as test substance, the tests may be substituted by the tests using the relevant substance as test substance by attaching such explanation.

(ii) In the case of major metabolites, etc. of active ingredients and if it is deemed necessary to conduct tests separately, the relevant major metabolites, etc. shall be test substances.

(iii) In cases where labeled compound is test substance, labeled nuclide shall be ^{14}C and labeled position shall be a stable site against metabolism, in principle. When molecular cleavage is expected, it is desirable to adopt as test substance multiple compounds that are labeled in different positions in the molecule for the purpose of understanding the metabolism of the cleavage substance.

④ The details of the concept of test substance for respective test items shall be specified as follows:

(i) Regarding acute inhalation toxicity test

Formulation itself shall be adopted as test substance, in principle. However, in the case of smoking agent, fog generated according to its application method shall be used as test substance, and in the case of fumigant, fumigation gas generated according to its application method shall be used as test substance.

(ii) Regarding skin irritation test and eye irritation test

The phrase “If formulation is difficult to use” shall refer to an agricultural chemical in the form of plate where its active ingredients are kneaded into resin, or a corrosive agricultural chemical, for example.

(iii) Regarding test on behavior in aerobic soil and test on behavior in anaerobic soil among tests on behavior in soil

Major transformation products for which the relevant tests on behavior are required shall mean metabolites that are generated 10% or more of applied amount and whose estimated half-life is 100 days or longer.

However, in cases where they can be deemed safe judging from the mobility and the degree of biological availability because of strong soil sorption, etc., or in cases where the toxicity is obviously low, this shall not apply.

More specifically,

(a) Tests on behavior in aerobic soil using the relevant metabolites shall be conducted in the case of metabolites that were generated 10 % or more of applied amount and whose estimated half-life was judged to be 100 days or longer on the basis of the test results regarding behavior in flooded aerobic soil using active ingredients.

Even in this case, if tests on behavior in aerobic soil using active ingredients were conducted, and the behavior of the relevant major transformation products can be confirmed from them, it is not necessary to conduct tests on behavior in aerobic soil using the relevant major metabolites.

(b) Tests on behavior in anaerobic soil using the relevant metabolites shall be conducted in the cases of metabolites that were generated 10 % or more of applied amount and whose estimated half-life was judged to be 100 days or longer and whose mobility in soil was judged to be not low on the basis of the test results regarding behavior in

aerobic soil using active ingredients.

Even in this case, if tests on behavior in anaerobic soil using active ingredients were conducted, and the behavior of the relevant major transformation products can be confirmed from them, it is not necessary to conduct tests on behavior in anaerobic soil using the relevant major transformation products.

(iv) Regarding bee toxicity test, silkworm toxicity test and natural enemy insect, etc. toxicity test

(a) In the case of acute oral toxicity test and contact toxicity test in bee toxicity test (2-8-1), acute dose oral toxicity test in silkworm toxicity test (2-8-2) and natural enemy insect, etc. toxicity test (2-8-3), TGAI shall be used as test substance, in principle.

(b) In the case of field test in bee toxicity test, residual toxicity test in silkworm toxicity test and field test in natural enemy insect, etc. toxicity test, formulation shall be used as test substance.

(c) When formulation is used as test substance, the one which gives the strongest effects in light of the formulation type, application method, application dosage(concentration), etc., shall be adopted.

(v) Regarding test on the properties, stability, degradability, etc. of active ingredients

(a) Active ingredients in their pure substance shall be used for tests, in principle. However, in the case of an agricultural chemical whose active ingredients are salt, ester, etc. and if reasonable explanation can be made, substance different from the active ingredients may be used as test substance. In the case of bioconcentration test, TGAI may be used for the test.

(b) The phrase “if it is difficult to use active ingredients in their pure state” shall refer to cases in which active ingredients in their pure substance cannot be obtained because it is difficult to purify TGAI. Besides this, if the purity of the relevant TGAI is comparatively high and if it is expected that test results using the relevant TGAI will not be different from those using active ingredients in their pure substance in light of the properties of the tests, the test results using the relevant TGAI may substitute for the test results using active ingredients in their pure substance.

(c) The phrase “Active ingredients, etc.” shall mean substances that are active ingredients and metabolites or degradation products generated by dissociation, degradation, metabolism, etc. of active ingredients within an organism or in the environment and that may affect humans and livestock or the environment (metabolites or degradation products, etc. that are subject to regulation in the evaluation of test on water polluting properties and test on residues in soil and that exceed residue of active ingredients during the degradative dissipation process of active ingredients).

(d) Test items required regarding the metabolites or degradation products of active ingredients shall be vapor pressure test, test on solubility in water, n-octanol/water partition coefficient test (excluding cases where solubility in water is 10mg/L or larger), soil adsorption test (excluding cases where solubility in water is 10mg/L or smaller), hydrolysis test (excluding cases where solubility in water is 10mg/L or smaller), test on photolysis in water (excluding cases where there is no absorbance in the range from 280 to 800nm), and bioconcentration test (excluding cases where solubility in water is 10mg/L or larger, or n-octanol/water partition coefficient is less than 3.5), in principle.

In addition, in the case of hydrolysis test and test on photolysis in water, if it is possible to measure metabolites or degradation products by the test using active ingredients, the relevant tests to be conducted independently for metabolites or degradation products may be omitted.

(e) In cases where active ingredients consist of multiple substances, tests shall be

conducted for each substance, but if it is extremely difficult to separate constituent substances (e.g. pyrethroid, or an agricultural chemical the active substances of which consist of enantiomer), representative substances or mixed substances may be used as test substance.

(2) Applicable crops

Applicable crops as referred to in Section I, paragraph (1) and (2) of the Notification by the Director-General shall be those listed in Appendix Tables 1-1 and 1-2, in principle. Crops as referred to in Section I, paragraph (4) of the Notification by the Director-General shall be those listed in Appendix Table 1-1, in principle.

In addition, the names of applicable crops listed in the left column of Appendix Tables 1-1 and 1-2 shall include the names of applicable crops listed in the right column, and those names listed in Appendix Tables 1-1 and 1-2 shall be used as the names of crops to be used in the application for registration, in principle.

(3) The number of trials for efficacy test and phytotoxicity test

Because efficacy and phytotoxicity of agricultural chemical are subject to annual climate change, differences in weather conditions among regions, agricultural cultivation practices, etc., efficacy test and phytotoxicity test shall be conducted considering these circumstances.

Therefore, efficacy test and phytotoxicity test pertaining to the test results to be submitted with the application for registration shall be conducted over at least two years at three or more facilities located in different prefectures, and the total number of trials shall be six or more, in principle. In this case, the tests conducted at the same facility shall be deemed to be each different one trial if they are conducted in different years.

In cases where the applicable crop pertaining to an application is a crop group, the conditions specified in Appendix Table 2 shall be applied.

In either one of the cases as listed in paragraph (1) to (5) in the "Number of trials" column in the Appended Table 1 of the Notification by the Director-General, the number of trials may be reduced as specified below. However, in the case of application for new registration, even if the conditions for reduction of the number of trials are met, this shall not apply, when the composition etc. of inert ingredients of the relevant agricultural chemical is different from those of the registered agricultural chemical that is the grounds for reduction of the number of trials and when there is a risk that the relevant inert ingredients may affect the efficacy and phytotoxicity of the relevant agricultural chemical.

The registered agricultural chemical as referred to herein shall be limited to agricultural chemicals registered by the applicant, and this shall not apply to cases where the applicant has no rights regarding the use of the relevant test results of the registered agricultural chemical.

If the conditions necessary for concluding tests are valid are not satisfied, the relevant tests shall not be treated as those eligible for application for registration.

① The details of paragraph (1) in the "Number of trials" column in the Appended Table 1 of the Notification by the Director-General shall be specified as follows:

(i) As for active ingredients relevant to a registered agricultural chemical ("registered agricultural chemical" shall mean an agricultural chemical that is currently being registered; the same shall apply hereinafter), the test results regarding efficacy and phytotoxicity concerning the formulation type pertaining to that registration have already been submitted for each combination of applicable crops and applicable diseases, insect pests or weeds, and the evaluations for registration have been conducted on its efficacy and phytotoxicity.

Therefore, the number of the trials for preparation of test results regarding efficacy and phytotoxicity to be submitted by the applicant for the following applications (a) and (b) shall be three or more and the trials shall be conducted at three or more facilities located in different prefectures, in principle and cases where all the trials are conducted in a single year may be acceptable.

(a) An application for registration pursuant to Article 2, paragraph (2) of the Law in the cases listed in item (i) to (iii) of paragraph (1) in the "Number of trials" column in the Appended Table 1 of the Notification by the Director-General, and

(b) An application for registration of change pursuant to Article 6-2, paragraph (1) of the Law in the cases listed in item (iv) and (v) of paragraph (1) in the "Number of trials"

column in the Appended Table 1 of the Notification by the Director-General;

(ii) The phrase "the combination of applicable crops and applicable diseases, insect pests or weeds pertaining to an application is the same as that of a registered agricultural chemical" shall mean cases where the combination of applicable crops and applicable diseases, insect pests or weeds pertaining to an application is the same as that of a registered agricultural chemical, or it is within the scope of that of a registered agricultural chemical.

(iii) The specific guidelines for item (i) to (v) of paragraph (1) in the "Number of trials" column in the Appended Table 1 of the Notification by the Director-General, shall be specified as follows:

(a) Regarding item (i)

[a] The term "formulation type" as referred to therein shall refer to dustable powder, dustable powder DL, dustable poweder FD, granule, dust-granule mixture, microgranule, microgranule F, powder, wettable powder, flowable formulation, dry flowable formulation, water dispersible granule, wettable poweder SE, emulsifiable concentrate, emulsifiable concentrate EW, emulsifiable concentrate CE, liquid formulation, liquid formulation ME, oil solution, aerosol, microcapsule, paste, smoking agent, painting formulation, ultra low volume spray formulation, fertilizer containing pesticide, etc. (the same shall apply hereinafter). Spray for home gardening using undiluted solution which is produced by diluting a registered agricultural chemical to the registered application concentration shall be deemed to be the same formulation type as the registered agricultural chemical.

[b] Cases where the active ingredients, combination of applicable crops and applicable diseases, insect pests or weeds, and the application method of the agricultural chemical pertaining to an application are the same as those of a registered agricultural chemical, shall fall under this case.

(b) Regarding item (ii)

[a] Cases where the active ingredients, the formulation type, the combination of applicable crops and applicable diseases, insect pests or weeds, and the application method of the agricultural chemical pertaining to an application are the same as those of a registered agricultural chemical, but the content of the active ingredients is changed and the amount of the active ingredients applied is smaller than that of the registered agricultural chemical, shall fall under this case.

[b] Cases where the active ingredients of a registered agricultural chemical consist of multiple optical isomers, and the abundance ratio of each optical isomer is changed to reduce the content of the active ingredients shall fall under this case.

(c) Regarding item (iii)

Cases where the agricultural chemical pertaining to an application contains active ingredients that are included in two or more registered agricultural chemicals and where its combination of applicable crops and applicable diseases, insect pests or weeds, and its application method are the same as those of the respective registered agricultural chemicals, but the content of the respective active ingredients of the agricultural chemical pertaining to the application is different from that of the respective registered agricultural chemicals, shall fall under this case.

(d) Regarding item (iv)

Cases where the application concentration or the application dosage (i.e. the amount of active ingredients applied) of a registered agricultural chemical is reduced but its combination of applicable crops and applicable diseases, insect pests or weeds is not changed, shall fall under this category.

(e) Regarding item (v)

Cases where the application method of a registered agricultural chemical is changed (e.g. from pricking-in hole treatment to plant foot treatment) shall fall under this case.

② The details of paragraph (2) in the "Number of trials" column in the Appended Table 1 of the Notification by the Director-General shall be specified as follows:

(i) As for active ingredients relevant to a registered agricultural chemical, the test results

regarding efficacy and phytotoxicity concerning the formulation type pertaining to that registration have already been submitted for each combination of applicable crops and applicable diseases, insect pests or weeds, and the evaluations for registration have been conducted on its efficacy and phytotoxicity.

Therefore, the number of the trials for preparation of test results regarding efficacy and phytotoxicity to be submitted by the applicant for the following applications (a) and (b) shall be two or more and the trials shall be conducted at two or more facilities located in different prefectures, in principle and cases where all the trials are conducted in a single year may be acceptable.

(a) An application for registration pursuant to Article 2, paragraph (2) of the Law in the cases listed in item (i) to (ii) of paragraph (2) in the "Number of trials" column in the Appended Table 1 of the Notification by the Director-General, and

(b) An application for registration of change pursuant to Article 6-2, paragraph (1) of the Law in the cases listed in item (iii) of paragraph (2) in the "Number of trials" column in the Appended Table 1 of the Notification by the Director-General;

(ii) The phrase "the combination of applicable crops and applicable diseases, insect pests or weeds pertaining to an application is the same as that of a registered agricultural chemical" shall mean cases where the combination of applicable crops and applicable diseases, insect pests or weeds pertaining to an application is the same as that of a registered agricultural chemical, or it is within the scope of that of a registered agricultural chemical.

(iii) The specific guidelines for item (i) to (iii) of paragraph (2) in the "Number of trials" column in the Appended Table 1 of the Notification by the Director-General, shall be specified as follows:

(a) Regarding item (i)

Cases where the active ingredients, the formulation type, the combination of applicable crops and applicable diseases, insect pests or weeds, and the application method of the agricultural chemical pertaining to an application are the same as those of a registered agricultural chemical, but the content of the active ingredients is changed and the amount of the active ingredients applied is either the same as or greater than that of the registered agricultural chemical, shall fall under this case.

(b) Regarding item (ii)

Cases where the agricultural chemical pertaining to an application contains active ingredients that are included in two or more registered agricultural chemicals and where the content of the respective active ingredients, its combination of applicable crops and applicable diseases, insect pest or weeds, and its application method is the same as those of the respective registered agricultural chemicals, shall fall under this case.

(c) Regarding item (iii)

Cases where the application concentration or the application dosage (i.e. the amount of active ingredients applied) of a registered agricultural chemical is increased, but its combination of applicable crops and applicable diseases, pests or weeds is not changed, and where aerial application, unmanned helicopter application, non-heat type fogging or low-volume liquid ground application is added to its application method, shall fall under this category.

③ The details of paragraph (3) in the "Number of trials" column in the Appended Table 1 of the Notification by the Director-General shall be specified as follows:

(i) As for active ingredients pertaining to a registered agricultural chemical, the test results regarding efficacy and phytotoxicity concerning the formulation type pertaining to that registration have already been submitted for each combination of applicable crops and applicable diseases, insect pests or weeds, and the evaluations for registration have been conducted on its efficacy and phytotoxicity.

Therefore, the number of the trials for preparation of test results regarding efficacy and phytotoxicity to be submitted by the applicant for the following applications (a) and (b) shall be two or more and the trials shall be conducted at two or more facilities located in different prefectures, in principle and cases where all the trials are conducted in a

single year may be acceptable.

(a) An application for registration pursuant to Article 2, paragraph (2) of the Law in the cases listed in item (iii), (iv) and (vi) of paragraph (3) in the "Number of trials" column in the Appended Table 1 of the Notification by the Director-General, and

(b) An application for registration of change pursuant to Article 6-2, paragraph (1) of the Law in the cases listed in item (i) to (vi) of paragraph (3) in the "Number of trials" column in the Appended Table 1 of the Notification by the Director-General;

However, in the cases listed in item (iii) to (v) of paragraph (3) in the "Number of trials" column in the Appended Table 1 of the Notification by the Director-General, the state of cultivation of crops and occurrence status of diseases, pests, weeds, etc. shall be considered.

(ii) The specific guidelines for item (i) to (vi) of paragraph (3) in the "Number of trials" column in the Appended Table 1 of the Notification by the Director-General, shall be specified as follows:

(a) Regarding item (i)

Cases where, when applicable diseases, pests, weeds etc. of a registered agricultural chemical are controlled with the registered agricultural chemical, other diseases, pests, weeds etc. that are not main to the relevant crops are controlled at the same time, and where those diseases, insect pests, weeds, etc. that are not main are added to applicable diseases, insect pests, weeds etc. of the registered agricultural chemical, fall under this case.

(b) Regarding item (ii)

This provision shall apply to cases where, within each of the Brassicaceae, the Solanaceae, the Cucurbitaceae, the Leguminosae, the Apiaceae, the Liliaceae (limited to *Allium*), or the Rosaceae (limited to fruit trees), a similar crop is added to the applicable crops for certain applicable diseases or pests. For example, cases where aphid for Japanese radish (*daikon*) is added while aphid for napa cabbage (*Brassica rapa* var. *pekinensis*) is already registered shall fall under this case.

(c) Regarding item (iii)

Cases where an application for registration (including an application for registration of change) is made for crops other than those listed in the Appendices Table 3-1 and Table 3-2 or is made for crops which are only grown in limited areas, shall fall under this case.

(d) Regarding item (iv)

The phrase "diseases, insect pests, or weeds that only occur in limited areas" shall refer to cases where the distribution of occurrence of diseases, pests or weeds is localized. This shall also include cases where the distribution of occurrence of diseases or insect pests is not localized, but the areas that require control of diseases or insect pests are limited.

(e) Regarding item (v)

Cases where there is a situation in which diseases, insect pests, or weeds that may give serious damages to agricultural production must be controlled emergently, and there are only a few effective registered agricultural chemicals against the said diseases, pests, or weeds, shall fall under this case.

(f) Regarding item (vi)

In the case of a spreader, the applicable scope of it shall be combinations of the applicable crops and the applicable agricultural chemicals. However, in cases where the applicable agricultural chemicals are herbicides that kill plants irrespective of crops and weeds (hereinafter referred to as "nonselective herbicide"), the applicable scope of the spreader shall be combinations of the applicable weeds and the applicable agricultural chemicals. Tests shall be conducted at 2 or more facilities for each applicable crop or applicable weed, and tests shall be conducted at 1 or more facilities for each combination of applicable crops and applicable agricultural chemicals, or for each combination of applicable weeds and applicable agricultural chemicals.

In cases other than the case where it is necessary to individually determine applicable crops and applicable agricultural chemicals, such as when the property of the spreader is adhesive such as paraffin or the like, and when the applicable agricultural chemical is herbicide (excluding nonselective herbicide) or plant growth regulator, etc.,

tests shall be conducted for each applicable crop group and each applicable agricultural chemical group as follows:

[a] Tests shall be conducted on 2 or more representative crops selected from among one of the following crop groups: “rice, cereals (others), cereal grains”, “vegetables, pulses (seeds), tuber crops, flowering plants and ornamental foliage plants”, “fruit trees” or “trees and shrubs”.

[b] Tests shall be conducted on 2 or more representative formulations selected from among one of the following agricultural chemical groups: “insecticide”, “fungicide” or “nonselective herbicide”.

④ The details of paragraph (4) in the “Number of trials” column in the Appended Table 1 of the Notification by the Director-General shall be specified as follows:

(i) As for active ingredients pertaining to a registered agricultural chemical, the test results regarding efficacy and phytotoxicity concerning the formulation type pertaining to that registration have already been submitted for each combination of applicable crops and applicable diseases, insect pests or weeds, and the evaluations for registration have been conducted on its efficacy and phytotoxicity.

Therefore, the number of the trials for preparation of test results regarding efficacy and phytotoxicity to be submitted by the applicant for the following applications (a) and (b) shall be three or more and the trials shall be conducted at three or more facilities located in different prefectures, in principle and cases where all the trials are conducted in a single year may be acceptable.

(a) An application for registration pursuant to Article 2, paragraph (2) of the Law in the cases listed in item (i) of paragraph (4) in the “Number of trials” column in the Appended Table 1 of the Notification by the Director-General, and

(b) An application for registration of change pursuant to Article 6-2, paragraph (1) of the Law in the cases listed in item (i) to (iii) of paragraph (4) in the “Number of trials” column in the Appended Table 1 of the Notification by the Director-General;

(ii) The specific guidelines for item (i) to (iii) of paragraph (4) in the “Number of trials” column in the Appended Table 1 of the Notification by the Director-General, shall be specified as follows:

(a) Regarding item (i)

Cases where the agricultural chemical pertaining to an application contains new active ingredients as well as the active ingredients of a registered agricultural chemical, and when the combination of applicable crops and applicable diseases, insect pests or weeds relevant to the active ingredients of the registered agricultural chemical among all combinations pertaining to the application and its application method are the same as those of the registered agricultural chemical, and when tests are conducted only for the combination relevant to the active ingredients of the registered agricultural chemical among all combinations pertaining to the application, shall fall under this case.

Examples: A mixture formulation in which a new type of active ingredient (fungicide) and an active ingredient (insecticide) of a registered agricultural chemical are mixed;
A mixture formulation in which a new type of active ingredient (insecticide) and an active ingredient (fungicide) of a registered agricultural chemical are mixed.

(b) Regarding item (ii)

The common diseases or insect pests difficult to control in many different crops shall include the following:

Examples: Tobacco cutworm (*Spodoptera litura*), beet armyworm (*Spodoptera exigua*), violet root rot, white root rot

(c) Regarding item (iii)

A registered agricultural chemical which is used without crops or in a state of not having contact with crops (excluding an agricultural chemical against diseases or pests that generates its effects by its systemic action into plant body) shall include the following:

Examples: An agricultural chemical that acts directly against diseases or pests in

soil (e.g. nematodes, cutworms);
An agricultural chemical that is used by placing it in certain locations, such as in the case of repellents, rodenticides and molluscicides.

⑤ The details of paragraph (5) in the "Number of trials" column in the Appended Table 1 of the Notification by the Director-General shall be specified as follows:

In cases where diseases or pests are controlled after relevant crops are harvested and moved into warehouses, silos, tents, etc., efficacy and phytotoxicity are not affected by the cultivation methods, etc. of the crops.

Therefore, the number of trials for preparation of the test results regarding efficacy and phytotoxicity, to be submitted by the applicant, for the application for registration of an agricultural chemical to be used in warehouses, silos, etc. or for the application for registration of change of such, shall be three or more, and the tests shall be conducted at three or more facilities, and cases where all the trials are conducted in a single year may be acceptable.

⑥ For an invertebrate used as biological control agent (macrobial pesticides), the name of the applicable crops may be "vegetables" or "fruit trees". The number of trials shall be 10 or more in total as follows: trials on one crop in the relevant crop group shall be conducted at three or more facilities located in different prefectures over two years; and trials on other two crops in the relevant crop group shall be conducted at two or more facilities located in different prefectures. In addition, the test results regarding efficacy and phytotoxicity of "vegetables" may be used as the test results regarding efficacy and phytotoxicity of "pulses (seeds)" and "tuber crops".

⑦ For an agricultural chemical used for soil fumigation (limited to those which are used when crops are not present and crops are seeded or planted after their volatilization to avoid phytotoxicity to the crops) which has already been registered for diseases, pests or weeds in three crops in "cereal grains", "cereals(others)", "vegetables", "tuber crops", "beans (seeds)", or "flowering plants and ornamental foliage plants", the test results regarding efficacy/phytotoxicity pertaining to that registered diseases, pests or weeds and three crops may be used to add that diseases, pests, or weeds to another crop of that crop group.

(4) The number of trials for limit test for phytotoxicity, test on residual odor in tea, and tobacco taste test

The details of the number of trials as referred to in the Appendix Table 1 of the Notification by the Director-General shall be specified as follows.

Tests conducted at the same facility shall be deemed to be each different one trial if they are conducted in different years.

Tests conducted in the same facility during the same period, where only the variety of test crops is changed, shall be deemed to be one trial.

In cases where the applicable crop pertaining to an application is a crop group, the conditions specified in the Appendix Table 2 shall be applied except in the case of herbicides and plant growth regulators, in principle.

(5) The number of trials and test facility standards in the case of test on residues in crops

The details of the number of trials and test facility standards pertaining to test on residues in crops as referred to in the Appendix Table 1 of the Notification by the Director-General shall be specified as follows:

① In cases where the applicable crop pertaining to an application is a crop group, the conditions specified in the Appendix Table 4 shall be applied.

② The phrase "a crop whose production volume is particular high" shall refer to crops listed in Appendix Table 3-1 that fall under any of the following items:

(i) A crop whose annual production is more than 300,000 tons in statistics etc. concerning the production volume or shipping volume of the crop (excluding crops whose major cultivation areas are biased toward certain areas.);

(ii) A crop whose annual production is more than 30,000 tons and 300,000 tons or less in statistics etc. concerning the production volume or shipping volume of the crop, and whose daily intake as agricultural products accounts for more than 1 % of the daily intake of agricultural, livestock and fishery products (excluding crops whose major cultivation areas are biased toward certain areas.);

③ The phrase "a crop whose production volume is high" shall refer to crops listed in Appendix Table 3-2 that fall under any of the following items:

(i) A crop whose annual production is more than 300,000 tons in statistics etc. concerning the production volume or shipment volume of the crop and whose major cultivation areas are biased toward certain areas;

(ii) A crop whose annual production is more than 30,000 tons and 300,000 tons or less in statistics etc. concerning the production volume or shipping volume of the crop, and whose daily intake as agricultural products accounts for more than 1% of the daily intake of agricultural, livestock and fishery products, and whose major cultivation areas are biased toward certain areas;

(iii) A crop whose annual production is more than 30,000 tons and 300,000 tons or less in statistics etc. concerning the production volume or shipping volume of the crop, and whose daily intake as agricultural products accounts for 1 % or less of the daily intake of agricultural, livestock and fishery products;

④ The phrase "a crop whose production volume is low" shall refer to crops other than those listed in the Appendix Tables 3-1 and 3-2.

⑤ The phrase "a crop whose production volume is particularly low" shall refer to crops whose annual production is estimated to be not more than 3,000 tons on the basis of the prefectural statistics etc. concerning the production volume or shipping volume of the crops.

⑥ In cases where the applicable crop pertaining to an application is a crop group and when the residue of the relevant agricultural chemical in crops included in that crop group is assumed to be extremely low or nil, crop group names and test crops specified in the Appendix Table 5 shall be applied when tests on residue of that agricultural chemical are conducted. One of the examples where the residue is assumed to be extremely low or nil is a herbicide applied to stems or leaves of weeds in a fruit farm.

⑦ For each crop in a crop group, in cases where its cultivation form is different and it is expected the difference in the residue in the crop is observed due to the difference in the cultivation form, sample preparation shall be conducted based on the cultivation method by which the residue is expected to be higher.

⑧ In the case of tests on residues of agricultural chemical whose applicable crops are crops listed in the left column in the Appendix Table 6, crops listed in the corresponding right column in the same table shall be used as test crop.

⑨ The details of "environmental conditions, sampling portion or other factors" as referred to in item ⑥ of the "Test facility standards" column in the case of tests on residues in crops in the Appendix Table 1 of the Notification by the Director-General shall be specified as follows. In addition, for the time being, the trials which may be conducted outside Japan shall only relate to the crop to be cultivated indoors (excluding cases where soil treatment is performed)and pasture grass of the grass family, pasture grass of pea family and sorghum. However, with regard to test results conducted outside Japan for pasture grass of the grass family, pasture grass of pea family and sorghum, the applicant may use them for the purpose of application for registration of an agricultural chemical only if the applicant submits the test results of three or more trials conducted in Japan and if the applicant submits all the test results obtained from tests conducted outside Japan under the same use pattern as those conducted in Japan until the application for registration is made.

(i) Environmental conditions (temperature, humidity, sunshine etc.);

(ii) Cultivation method (including cropping type, cultivation matrix);

(iii) Application method of agricultural chemical (including application implement);

(iv) Method for harvesting crops (including harvesting implement);

(v) Size of harvested crops (size of shipping crops);

(vi) Sampling portion;

⑩ When the test results of six or more trials regarding residues in crops are to be submitted, and in the case of falling under any of the following items (i) and (ii), the test results regarding residues in crops concerning other agricultural chemical specified respectively in the following items may be submitted in substitution for the relevant test results.

(i) When other agricultural chemical has the same active ingredients and formulation type as the agricultural chemical pertaining to an application, and when the difference in the use pattern compared to the relevant agricultural chemical falls under any of the following items (a) to (c); The test results regarding residues in crops concerning the other agricultural chemical.

(a) A difference in the application dosage (the amount of the active ingredients applied) or application concentration (the concentration of the active ingredients applied) is within ±25 %. (The case where it corresponds to (b) or (c) is excluded.)

(b) A difference in the application number is within ±25 %. (The case where it corresponds to (a) or (c) is excluded.)

(c) A difference in the pre-harvest interval is within ±25 %. (The case where it corresponds to (a) or (b) is excluded.)

(ii) When the agricultural chemical pertaining to an application is a formulation type which is diluted with water prior to spray (excluding sustained release formulations, such as microcapsule), and when it is used eight or more days before the harvest date; The test results regarding residues in crops which are submitted or have been submitted with an application for registration of other agricultural chemical (limited to those diluted with water prior to spray; however, sustained release formulations, such as microcapsule, are excluded.) which has the same active ingredients as the agricultural chemical pertaining to said application, but is a different formulation type.

(6) Number of trials for test on metabolism in plants

The details of the "Number of trials" column as referred to in the Appended Table 1 in the Notification by the Director-General shall be specified as follows:

① In principle, for each plant category which the applicable crops pertaining to an application belong to, tests shall be conducted on 1 or more types of crops selected from among the crops in the plant category. However, if it is shown that there is not a large difference in metabolism among crops involved in each of three types of plant categories including a plant category not relevant to the application, no additional tests are required regardless of the presence of the test results concerning the plant category pertaining to the application.

② If the applicable crops pertaining to an application are limited to only one plant category and the said plant category includes crops other than test crops, the test results concerning one different type of plant are required in addition to the test results concerning the said plant category.

③ If the applicable crops pertaining to an application are limited to 1 type of crop and the test plant is identical to the crop, no additional tests are required.

The details of the "Cases in which test results need not be submitted" column as referred to in the Appendix Table 2 in the Notification by the Director-General shall be specified as follows:

The phrase "a crop whose production volume is low" shall refer to crops other than crops listed in the Appendix Table 3-1 or Appendix Table 3-2 (The same shall apply hereinafter).

(7) Test facility standards

The details of the test facilities capable of adequate conduct of efficacy test, phytotoxicity test, test on derivation of predicted environmental concentration, test on residues in soil, and test on residues in crops in cases where the applicable crop is a crop whose production volume is low among tests on efficacy, phytotoxicity, toxicity, and persistence of agricultural chemicals in the Appendix Table 1 of the Notification by the Director-General shall be specified as follows.

Regarding limit test for phytotoxicity, test on phytotoxicity to adjacent crops, test on phytotoxicity to succeeding crops, test on toxicity on beneficial organisms other than aquatic animals and plants, and test on translocation to milk, no specific standards are established for test facilities; however, from the viewpoint of ensuring the reliability of the tests, it is desirable to conduct the tests by those who have a certain level of expert knowledge in such fields as crop cultivation and test organism handling.

① Regarding the test facilities capable of adequate conduct of efficacy test, and those capable of adequate conduct of phytotoxicity test

(i) Test facilities shall be any of the following facilities:
- Official test and research facilities, including incorporated administrative agencies, prefectural agricultural experimental stations, and national university corporations;
- Semi-official test and research facilities, including plant protection stations of Ministry of Agriculture, Forestry and Fisheries of Japan, prefectural plant disease and insect control stations, public interest corporations having expertise, private universities, and test and research facilities engaged exclusively in studies on cultivation management of industrial crops; and
- Private test facilities that meet the following conditions:
  (a) Operating procedures for test on efficacy and phytotoxicity shall be documented,
  (b) In principle, the study plan, field notes and final report shall be maintained until the agricultural chemical is registered,
  (c) Equipment and instruments necessary for test on efficacy and phytotoxicity shall be put into place and,
  (d) An organizational structure shall be established for adequate conduct of cultivation management of crops and test on efficacy and phytotoxicity.

(ii) Regarding field test etc., cases where the study personnel of the test facility falling under the preceding item (i) borrows fields etc. from farmers temporarily for a period necessary to conduct the test and conducts the test shall fall under this case.

② Regarding the test facilities capable of adequate conduct of test on derivation of predicted environmental concentration, and those capable of adequate conduct of test on residues in soil

(i) Test facilities shall be any of the following facilities:
- Official test and research facilities, including incorporated administrative agencies, prefectural agricultural experimental stations, and national university corporations; and
- Semi-official test and research facilities, including public interest corporations having expertise, private universities, and test and research facilities engaged exclusively in studies on cultivation management of industrial crops.

(ii) Regarding field test etc., cases where the study personnel of the test facility falling under the preceding item (i) borrows fields etc. from farmers temporarily

for a period necessary to conduct the test and conducts the test shall fall under this case.

(iii) Regarding analytical test, even in cases where the applicant or farmers conducted the test, when the study personnel of the test facility falling under the preceding item (i) designed and directed or evaluated the test, the test results shall be handled as those obtained from test conducted in official test and research facilities.

③ Regarding test facilities for test on residues in crops in cases where the applicable crop is a crop whose production volume is low

(i) Test facilities shall be any of the following facilities:
- Official test and research facilities, including incorporated administrative agencies, prefectural agricultural experimental stations, and national university corporations; and
- Semi-official test and research facilities, including plant protection stations of Ministry of Agriculture, Forestry and Fisheries of Japan, prefectural plant disease and insect control stations, public interest corporations having expertise, private universities, and test and research facilities engaged exclusively in studies on cultivation management of industrial crops.

(ii) Regarding field test etc., cases where the study personnel of the test facility falling under the preceding item (i) borrows fields etc. from farmers temporarily for a period necessary to conduct the test and conducts the test shall fall under this case.

(iii) Regarding test on residues in crops whose production volume is low, when the tests were conducted by one other than the applicant who obtains a registration as a registered conformity assessment body according to Article 33 of the Food Sanitation Act (Act No. 233 of 1947), or who obtains a registration for a measurement certification business of concentration according to Article 107 of the Measurement Act (Act No. 51 of 1992), or who obtains a conformity certification for compliance with an international standard for laboratory accreditation, the test results shall be handled as those obtained from tests conducted in semi-official test and research facilities.

(8) Ensuring the reliability of test results

Regarding the test conducted by the test facilities capable of adequate conduct of efficacy test and those capable of adequate conduct of phytotoxicity test listed in the Appendix Table 1 of the Notification by the Director-General, the reliability of the test results shall be assured as follows:

① The applicant shall conduct a review of the test results by experts before application, in order to assure the reliability of the test results regarding efficacy and phytotoxicity. However, the review for test results concerning crops whose production volume is low may be omitted only if the tests were conducted by prefectural agricultural experimental stations or prefectural plant disease and insect control stations.

② In principle, the following documents shall be maintained until the relevant agricultural chemical is registered, so that questions can be confirmed to be solved when the test results regarding efficacy and phytotoxicity are questioned:

(i) Test operating procedures,

(ii) Study plan, field notes, and final report, and

(iii) Summary of review results by experts
(a) Organizer and attending experts in the review,
(b) Review date and venue, and
(c) Evaluation results

Section 3 Exceptions as regards submission of test results

Test results as listed in Section I of the Notification by the Director-General are indispensable to the evaluation for registration of agricultural chemicals, but in some cases, in view of the type of active ingredients, the formulation type, the application method etc. of the relevant agricultural chemical, the submission of some parts of test results may be unnecessary.

The conditions where the test results need not be submitted should be determined according to each agricultural chemical pertaining to an application, but in light of the position etc. of individual test results in the evaluation for registration, some of the concepts for cases where the test results need not be submitted are specified in the Appendix Table 2 in the Notification by the Director-General.

The guidelines for the Appendix Table 2 in the Notification by the Director-General and other exceptions as regards the submission of test results shall be specified as follows.

Cases where it is difficult to implement tests due to the properties etc. of the test substance shall be deemed as cases where there are "reasonable grounds on which the submission of some parts of test results is unnecessary" as cited herein.

(1) Test results regarding phytotoxicity

① Test results regarding phytotoxicity to the applicable crops

(i) Test results regarding residual odor in tea

In addition to cases where the applicable crops do not include tea, the following cases shall fall under the cases where the relevant test results need not be submitted, for example.

(a) When it is found that there is no risk that tea as the applicable crop will be directly exposed to the relevant agricultural chemical, in light of its formulation type, its application method etc.; For example, the following cases shall fall under this case:
[a] When the agricultural chemical is used with its components enclosed, such as in the case of attractants;
[b] When the agricultural chemical is used by placing it in certain locations, such as in the case of repellents, rodenticides and molluscicides;
(b) When the agricultural chemical is applied to soil (excluding cases where an agricultural chemical with systemic action into plant body is used);

(ii) Test results regarding tobacco taste

In addition to the cases where the applicable crops do not include tobacco, the following cases shall fall under the cases where the relevant test results need not be submitted, for example.

(a) When it is found that there is no risk that tobacco as the applicable crop will be directly exposed to the relevant agricultural chemical, in light of its formulation type, its application method etc.; For example, the following cases shall fall under this case:
[a] When the agricultural chemical is used with its components enclosed, such as in the case of attractants;
[b] When the agricultural chemical is used by placing it in certain locations, such as in the case of repellents, rodenticides and molluscicides;
(b) When the agricultural chemical is applied to soil (excluding cases where an agricultural chemical with systemic action into plant body is used);
(c) When the agricultural chemical is used in a nursery bed;
(d) When the agricultural chemical is applied by attaching it directly to seeds etc., such as in the case of dust coating;

(iii) Test results regarding critical dosage (or concentration) for phytotoxicity

Regarding the phrase "When it is found that there is no risk that the applicable crops will be exposed to the relevant agricultural chemical beyond its applicable range (application dosage, concentration), in light of its application method etc.;", the following

cases shall fall under this case:

(a) When the agricultural chemical is used with its components enclosed, such as in the case of attractants;

(b) When the chemical is used by placing it in certain locations, such as in the case of repellents, rodenticides and molluscicides;

② Test results regarding phytotoxicity to adjacent crops

(i) Test results regarding phytotoxicity due to drift and scattering

Regarding the phrase “When it is found that there is no risk that the relevant agricultural chemical will affect adjacent crops (i.e. phytotoxicity) through its drifting and scattering, in light of the type of its active ingredients, its formulation type, its application method etc.;”, the following cases shall fall under this case:

(a) When the agricultural chemical has already been widely used as spreader and it has been confirmed that there is no problem in terms of phytotoxicity;

(b) When the agricultural chemical is used with its components enclosed, such as in the case of attractants;

(c) When the agricultural chemical is used by placing it in certain locations, such as in the case of repellents, rodenticides and molluscicides;

(d) When the relevant agricultural chemical is used only inside of a facility such as warehouse without the possibility of drifting to adjacent crops;

(e) When the agricultural chemical is applied to soil (excluding herbicides and soil fumigants); when it is applied to paddy water (throw-in method, dropping method, application at irrigation inlet); when it is applied to a nursery box; when it is applied by attaching it directly to seeds etc. such as in the case of dust coating; or when it is applied to the applicable crops by painting or trunk injection;

(f) When the formulation type is a granule (excluding herbicides to be used in places other than paddies, or when it is applied by aerial application or unmanned helicopter application);

(ii) Test results regarding phytotoxicity due to runoff from paddy water

Regarding the phrase “When it is found that there is no risk that the relevant agricultural chemical will affect adjacent crops (i.e. phytotoxicity) through its runoff from paddy water into water systems such as rivers, in light of its application method etc.;”, cases where agricultural chemicals other than herbicides are used shall fall under this case (excluding the case referred to in item (i) in the Appendix Table 2 of the Notification by the Director-General).

(iii) Test results regarding phytotoxicity due to volatilization

Regarding the phrase "When it is found that there is no risk that the relevant agricultural chemical will affect adjacent crops (i.e. phytotoxicity) through its volatilization, in light of the properties of its active ingredients, its formulation type, its application method etc.;”, cases where the following agricultural chemicals are used shall fall under this case:

(a) Agricultural chemicals other than herbicides;

(b) Herbicides in which the vapor pressure of the active ingredients is approximately less than 10^{-4} hPa

③ Test results regarding phytotoxicity to succeeding crops

Regarding the phrase “When it is found that there is no risk that the relevant agricultural chemical will affect crops (i.e. phytotoxicity) that are to be cultivated after the applicable crops, in light of its application method, the degree of its persistence in soil etc.;”, cases where the relevant agricultural chemical is an agricultural chemical, excluding soil-applied herbicides, in which the estimated half-life of its active ingredients does not exceed 100 days in principle on the basis of the test results regarding residues in soil shall fall under this case.

(2) Test results regarding toxicity

① Test results regarding acute oral toxicity

The test results need not be submitted in the following cases:

(i) Regarding tests using TGAI

When the active ingredients of the relevant agricultural chemical are found to be safe because they are of very low toxicity, in light of their type etc.; For example, cases where the active ingredients of the relevant agricultural chemical have already been used generally and widely for food etc., and well known to the public as safe shall fall under this case.

(ii) Regarding tests using formulation

When the relevant agricultural chemical is found to be safe because there is very little risk of direct oral ingestion of it, in light of its formulation type, its application method etc.; For example, cases where the relevant agricultural chemical is used with its components enclosed, such as in the case of attractants shall fall under this case.

② Test results regarding acute dermal toxicity

In addition to "when it is found that the relevant agricultural chemical is corrosive;", for example, the test results need not be submitted in the following cases:

(i) Regarding tests using TGAI

(a) When the active ingredients of the relevant agricultural chemical are found to be safe because they are of very low toxicity, in light of their type etc.; For example, cases where the active ingredients of the relevant agricultural chemical have already been used generally and widely for food etc., and well known to the public as safe shall fall under this case.

(b) When the relevant agricultural chemical is found to be safe because the amount of exposure (limited to transdermal exposure) to its components is extremely very small, in light of its formulation type, its application method etc.; For example, cases where the relevant agricultural chemical is used with its components enclosed, such as in the case of attractants shall fall under this case.

(ii) Regarding tests using formulation

When the relevant agricultural chemical is found to be safe because the amount of exposure (limited to transdermal exposure) to its components is extremely very small, in light of its formulation type, its application method etc.; For example, cases where the relevant agricultural chemical is used with its components enclosed, such as in the case of attractants shall fall under this case.

③ Test results regarding acute inhalation toxicity

The test results need not be submitted in the following cases:

(i) Regarding tests using TGAI

(a) When the active ingredients of the relevant agricultural chemical are found to be safe because they are of very low toxicity, in light of their type etc.; For example, cases where the active ingredients of the relevant agricultural chemical have already been used generally and widely for food etc., and well known to the public as safe shall fall under this case.

(b) When the relevant agricultural chemical is found to be safe because there is very little risk of direct exposure to its components through inhalation, in light of its formulation type, its application method etc.; For example, the following cases shall fall under this

case:

[a] When the relevant agricultural chemical is used with its components enclosed, such as in the case of attractants

[b] When the relevant agricultural chemical is used by placing it in certain locations such as in the case of repellents, rodenticides and molluscicides;

(ii) Regarding tests using formulation

Regarding the phrase "when it is found that there is no risk that users will be exposed to the relevant agricultural chemical through inhalation, in light of its formulation type, its application method etc.;", for example, cases where agricultural chemicals other than those to be used by gasifying their components, such as fumigants and smoking agents are used shall fall under this case.

④ Test results regarding skin irritation

In addition to "when it is found that the relevant agricultural chemical is corrosive;", the test results need not be submitted in cases where the relevant agricultural chemical is found to be safe because there is very little risk of direct exposure to it, in light of its formulation type, its application method etc. For example, cases where the relevant agricultural chemical is used with its components enclosed, such as in the case of attractants shall fall under this case.

⑤ Test results regarding eye irritation

In addition to the cases referred to in items (i) and (ii) in the Appendix Table 2 in the Notification by the Director-General, the test results need not be submitted in cases where the relevant agricultural chemical is found to be safe because there is very little risk of direct exposure to it, in light of its formulation type, its application method etc.; For example, cases where the relevant agricultural chemical is used with its components enclosed, such as in the case of attractants shall fall under this case.

⑥ Test results regarding skin sensitization

The test results need not be submitted in the following cases:

(i) Regarding tests using TGAI

(a) When the active ingredients of the relevant agricultural chemical are found to be safe because they are of very low toxicity, in light of their type etc.; For example, cases where the active ingredients of the relevant agricultural chemical have already been used generally and widely for food etc., and well known to the public as safe shall fall under this case.

(b) When the relevant agricultural chemical is found to be safe because the amount of exposure (limited to transdermal exposure) to its components is extremely very small, in light of its formulation type, its application method etc.; For example, cases where the relevant agricultural chemical is used with its components enclosed, such as in the case of attractants shall fall under this case.

(ii) Regarding tests using formulation

When the relevant agricultural chemical is found to be safe because the amount of exposure (limited to transdermal exposure) to its components is extremely very small, in light of its formulation type, its application method etc.; For example, cases where the relevant agricultural chemical is used with its components enclosed, such as in the case of

attractants shall fall under this case.

⑦ Test results regarding acute neurotoxicity

(i) Regarding the phrase "When it is found that there is no risk of neurotoxicity on the basis of the test results regarding acute toxicity etc.;", cases where it is confirmed that there is no finding to suggest specific neurotoxicity under sublethal dose on the basis of the general clinical observations in the acute oral toxicity test and the detailed clinical observations , a functional test, a histopathological examination, etc. in the 90-day repeated dose oral toxicity test using rats, and; cases where there is no similarity to any known neurotoxic chemicals in terms of chemical structure, or; cases where no neurotoxicity is suggested in the repeated dose oral neurotoxicity or the 28-day repeated dose oral neurotoxicity test (OECD424), shall fall under this case.

(ii) In addition to the cases specified in the preceding paragraph, the test results need not be submitted in the following cases:

(a) When the active ingredients of the relevant agricultural chemical are found to be safe because they are of very low toxicity, in light of their type etc.; For example, cases where the active ingredients of the relevant agricultural chemical have already been used generally and widely for food etc., and well known to the public as safe shall fall under this case.

(b) When the relevant agricultural chemical is found to be safe because the amount of exposure to its components is extremely very small, in light of its formulation type, its application method etc.; For example, the following cases shall fall under this case:

[a] When the relevant agricultural chemical is used with its components enclosed, such as in the case of attractants;

[b] When the relevant agricultural chemical is used by placing it in certain locations, such as in the case of repellents, rodenticides and molluscicides;

⑧ Test results regarding acute delayed neurotoxicity

(i) The phrase "When it is found that there is no risk of delayed neurotoxicity on the basis of the test results regarding acute toxicity etc.;" shall refer to cases where it is found that the active ingredients of the relevant agricultural chemical do not have cholinesterase inhibition activity on the basis of the test results regarding acute toxicity etc.

(ii) The phrase "When it is found that there is no risk of delayed neurotoxicity, in light of correlations with the chemical structures of chemicals that are known to have delayed neurotoxic effects;" shall refer to cases where agricultural chemicals other than those whose active ingredients are phosphoric ester and have cholinesterase inhibition activity are used.

(iii) In addition to the cases specified in the preceding two paragraphs, the test results need not be submitted in the following cases:

(a) When the active ingredients of the relevant agricultural chemical are found to be safe because they are of very low toxicity, in light of their type etc.; For example, cases where the active ingredients of the relevant agricultural chemical have already been used generally and widely for food etc., and well known to the public as safe shall fall under this case.

(b) When the relevant agricultural chemical is found to be safe because the amount of exposure to its components is extremely very small, in light of its formulation type, its application method etc.; For example, the following cases shall fall under this case:
[a] When the relevant agricultural chemical is used with its components enclosed, such as in the case of attractants;
[b] When the relevant agricultural chemical is used by placing it in certain locations, such as in the case of repellents, rodenticides and molluscicides;

⑨ Test results regarding 90-day repeated dose oral toxicity, teratogenicity, mutagenicity, pharmacology and metabolism in animals

(i) Regarding the phrase "When the relevant agricultural chemical is found to be safe because the amount of exposure to and ingestion of its components etc. is extremely very small when it is used, in light of its formulation type, its application method etc.;" for example, the following cases shall fall under this case:

However, the cases referred to in the following item (b) shall not apply to the reverse mutation test among the tests on mutagenicity. This is because the relevant test results are regarded as basic information on the toxicity of chemical substances including agricultural chemicals, so it is necessary to confirm the safety in terms of the reverse mutation.

(a) When the relevant agricultural chemical is used with its components enclosed, such as in the case of attractants;
(b) When the relevant agricultural chemical is used by placing it in certain locations, such as in the case of repellents, rodenticides and molluscicides;

In this case, "ingestion" shall mean oral ingestion of components etc. of the relevant agricultural chemical through crops.

(ii) Regarding the phrase "When the relevant agricultural chemical is found to be safe because its components etc. are of very low toxicity, in light of their type etc.;", for example, cases where the active ingredients of the relevant agricultural chemical have already been used generally and widely for food etc., and well known to the public as safe shall fall under this case.

However, this shall not apply to the reverse mutation test among tests on mutagenicity except in the case of substances that are used generally and widely as food and are found to be particularly safe such as nitrogen and starch. This is because the relevant test results are regarded as basic information on the toxicity of chemical substances including agricultural chemicals, so it is necessary to confirm the safety in terms of the reverse mutation.

(iii) In addition to the cases specified in the preceding two paragraphs, cases where the 90-day repeated inhalation toxicity test is conducted instead of the 90-day repeated dose oral toxicity test due to the high volatility etc. of the components in the relevant agricultural chemical such as in the case of fumigants shall fall under this case.

⑩ Test results regarding 21-day repeated dermal toxicity

(i) Regarding the phrase "When it is found that there is no risk of long-term percutaneous exposure to the relevant agriculture chemical on persons who are applying it;", for example, the following cases shall fall under this case:
(a) When the relevant agricultural chemical is used with its components enclosed, such as in the case of attractants;
(b) When the relevant agricultural chemical is used by placing it in certain locations, such

as in the case of repellents, rodenticides and molluscicides;

(ii) Regarding the phrase "When it is found that there is no risk that the relevant agricultural chemical has high dermal toxicity on the basis of the test results regarding acute dermal toxicity;", cases where it is found that there is no remarkably high dermal toxicity compared with the acute toxicity of other exposure paths on the basis of the test results regarding acute dermal toxicity shall fall under this case.

(iii) In addition to the cases specified in the preceding two paragraphs, when the active ingredients of the relevant agricultural chemical are found to be safe because they are of very low toxicity, in light of their type etc.; For example, cases where the active ingredients of the relevant agricultural chemical have already been used generally and widely for food etc., and well known to the public as safe shall fall under this case.

⑪ Test results regarding 90-day repeated inhalation toxicity

(i) Regarding the phrase "When it is found that there is no risk of long-term inhalation exposure to the relevant agricultural chemical on persons who are applying it", for example, the following cases shall fall under this case.

(a) When the relevant agricultural chemical is used with its components enclosed, such as in the case of attractants;

(b) When the relevant agricultural chemical is used by placing it in certain locations, such as in the case of repellents, rodenticides and molluscicides;

(ii) Regarding the phrase "When it is found that there is no risk that the relevant agricultural chemical has high inhalation toxicity on the basis of the test results regarding acute inhalation toxicity;", cases where it is found that there is no remarkably high inhalation toxicity compared with the acute toxicity of other exposure paths on the basis of the test results regarding acute inhalation toxicity shall fall under this case.

(iii) In addition to the cases specified in the preceding two paragraphs, when the active ingredients of the relevant agricultural chemical are found to be safe because they are of very low toxicity, in light of their type etc.; For example, cases where the active ingredients of the relevant agricultural chemical have already been used generally and widely for food etc., and well known to the public as safe shall fall under this case.

⑫ Test results regarding repeated dose oral neurotoxicity

(i) Regarding the phrase "When it is found that there is no risk of neurotoxicity on the basis of the test results regarding 90-day repeated dose oral toxicity etc.;", cases where it is confirmed that there is no finding to suggest specific neurotoxicity under sublethal dose on the basis of the detailed clinical observations, a functional test, a histopathological examination, etc. in the 90-day repeated dose oral toxicity test using rats etc., and; cases where there is no similarity to any known neurotoxic chemicals in terms of chemical structure, or; cases where no neurotoxicity is suggested in the 28-day repeated dose oral neurotoxicity test (OECD424), shall fall under this case.

(ii) In addition to the cases specified in the preceding paragraph, the test results need not be submitted in the following cases:

(a) When the active ingredients of the relevant agricultural chemical are found to be safe because they are of very low toxicity, in light of their type etc.; For example, cases where the active ingredients of the relevant agricultural chemical have already been used generally and widely for food etc., and well known to the public as safe shall fall under this case.

(b) When the relevant agricultural chemical is found to be safe because the amount of exposure to its components is extremely very small, in light of the formulation type, the application method etc. of the relevant agricultural chemical; For example, the following cases shall fall under this case:

[a] When the relevant agricultural chemical is used with its components enclosed, such

as in the case of attractants

[b] When the chemical is used by placing it in certain locations such as in the case of repellents, rodenticides and molluscicides

⑬ Test results regarding 28-day repeated administration delayed neurotoxicity

In addition to the cases where it is clearly found that there is no delayed neurotoxicity on the basis of the test results regarding acute delayed neurotoxicity, in cases where it is found that the test results regarding acute delayed neurotoxicity need not be submitted, the said test results need not be submitted.

⑭ Test results regarding 1-year repeated dose oral toxicity, carcinogenicity and reproductive toxicity

(i) Regarding the phrase "When the relevant agricultural chemical is found to be safe because there is no risk that humans will ingest its components etc. for a long period, or because the amount of ingestion of them would be extremely very small, in light of its formulation type, its application method etc.;", for example, the following cases shall fall under this case:

(a) When the relevant agricultural chemical is used for crops other than those that are to be used for food;

(b) When there is no risk that the applicable crops will be directly exposed to the relevant agricultural chemical, in light of its formulation type, its application method etc.; For example, the following cases shall fall under this case:

[a] When the relevant agricultural chemical is used with its components enclosed, such as in the case of attractants;

[b] When the relevant agricultural chemical is used by placing it in certain locations, such as in the case of repellents, rodenticides and molluscicides;

(c) When it is found that there is very little risk that humans will ingest the components etc. of the relevant agricultural chemical through the relevant crops because it is to be used in the early growth stage of the applicable crops such as in the case of an agricultural chemical applied by attaching it directly to seeds etc. like dust coating;

(ii) Regarding the phrase "When the relevant agricultural chemical is found to be safe because its components etc. are of very low toxicity, in light of their type etc.;", for example, cases where the active ingredients of the relevant agricultural chemical have already been used generally and widely for food etc., and well known to the public as safe shall fall under this case.

⑮ Test results regarding metabolism in plants

(i) Regarding the phrase "When the relevant agricultural chemical is found to be safe because there is no risk that humans will ingest its components etc. for a long period, or because the amount of ingestion of them would be extremely very small, in light of its formulation type, its application method etc.;", for agricultural chemicals other than those referred to in the item (i) "When the relevant agricultural chemical is used for crops other than those that are to be used for food (including industrial crops and crops to be used for animal feed);" in the Appendix Table 2 in the Notification by the Director-General, the following cases shall fall under this case:

(a) When there is no risk that the applicable crops will be directly exposed to the relevant agricultural chemical, in light of its formulation type, its application method etc.; For example, the following cases shall fall under this case:

[a] When the relevant agricultural chemical is used with its components enclosed, such as in the case of attractants;

[b] When the relevant agricultural chemical is used by placing it in certain locations, such as in the case of repellents, rodenticides and molluscicides;

(b) When the applicable crops are crops after harvesting, such as in the case of

agricultural chemical used for warehouse fumigation;

(c) When it is found that there is very little risk that humans will ingest the components etc. of the relevant agricultural chemical through the relevant crops because it is to be used in the early growth stage of the applicable crops such as in the case of an agricultural chemical applied by attaching it directly to seeds etc. like dust coating;

(ii) Regarding the phrase "When the relevant agricultural chemical is found to be safe because its components etc. are of very low toxicity, in light of their type etc.;", for example, cases where the active ingredients of the relevant agricultural chemical have already been used generally and widely for food etc., and well known to the public as safe shall fall under this case.

⑯ Test results regarding behavior in soil

(i) Regarding the phrase "When it is found that there is no risk that the components etc. of the relevant agricultural chemical will contaminate farmland soil where it is used, in light of its formulation type, its application method etc.;", the following cases shall fall under this case:

(a) When the relevant agricultural chemical is used with its components enclosed, such as in the case of attractants;

(b) When the relevant agricultural chemical is used by placing it in certain locations, such as in the case of repellents, rodenticides and molluscicides;

(c) When the relevant agricultural chemical is applied to the applicable crops by painting or trunk injection;

(d) When the relevant agricultural chemical is used only inside of a facility such as warehouse where soil is not exposed;

(e) When the relevant agricultural chemical is not used in large quantities over a wide area at one time, such as in the case of aerosol;

(ii) Regarding the phrase "When the relevant agricultural chemical is found to be safe because its components etc. are of very low toxicity, in light of their type etc.;", cases where the active ingredients of the relevant agricultural chemical have already been used generally and widely for food etc., and well known to the public as safe shall fall under this case.

(iii) "Paddies" shall include all states where crops are cultivated in a flooding condition and include cases where they are used for the cultivation of "*kuwai* (*Sagittaria trifolia* var. *edulis*)", "lotus root", "rush", etc. besides paddy rice.

(iv) Whether or not a case falls under the phrase "cases where the test results are considered necessary, in light of the dissipation rate of its components, etc. in flooded aerobic soil;" or the phrase "when it is found that the components etc. of the relevant agricultural chemical dissipate rapidly in aerobic soil;" shall be determined by judging whether the estimated half-life of the components etc. of the relevant agricultural chemical in soil exceeds 100 days or not, in principle.

(v) The phrase "its mobility in soil is low" shall mean cases where the aqueous solubility of the test substance is 10 mg/L or lower, or where the soil adsorption coefficient (K^{ads}_{Foc}) is 500 or larger, in principle.

⑰ Test results regarding behavior in water

(i) Regarding the phrase "When it is found that there is no risk that the components etc. of the relevant agricultural chemical will run off into water systems such as rivers, in light of its formulation type, its application method etc.;", the following cases shall fall under this case:

(a) When the relevant agricultural chemical is used with its components enclosed, such as in the case of attractants;

(b) When the relevant agricultural chemical is used by placing it in certain locations, such as in the case of repellents, rodenticides and molluscicides;

(c) When the relevant agricultural chemical is applied to the applicable crops by painting

or trunk injection;
(d) When the relevant agricultural chemical is used only inside of a facility such as warehouse and greenhouse;
(e) When the relevant agricultural chemical is not used in large quantities over a wide area at one time, such as in the case of aerosol;

(ii) Regarding the phrase "When the relevant agricultural chemical is found to be safe because its components etc. are of very low toxicity, in light of their type etc.;", for example, cases where the active ingredients of the relevant agricultural chemical have already been used generally and widely for food etc., and well known to the public as safe for humans, livestock, and aquatic animals and plants shall fall under this case.

(3) Test results regarding toxicity on aquatic animals and plants

① Test results regarding fish acute toxicity and *Daphnia spp* acute immobilization

(i) Regarding tests using TGAI

(a) Regarding the phrase "When it is found that there is no risk that the components etc. of the relevant agricultural chemical will run off into water systems such as rivers, in light of its formulation type, its application method etc.;", the following cases shall fall under this case:
[a] When the relevant agricultural chemical is used with its components enclosed, such as in the case of attractants;
[b] When the relevant agricultural chemical is applied to the applicable crops by painting or trunk injection;
[c] When the relevant agricultural chemical is used only inside of a facility such as warehouse and greenhouse;
[d] When the relevant agricultural chemical is not used in large quantities over a wide area at one time, such as in the case of aerosol;
[e] When the agricultural chemical is applied by attaching it directly to seeds etc., such as in the case of dust coating;

(b) Regarding the phrase "When the relevant agricultural chemical is found not to be harmful because its components etc. are of very low toxicity, in light of their type etc.;", for example, cases where the active ingredients of the relevant agricultural chemical have already been used generally and widely for food etc., and well known to the public as safe for aquatic animals shall fall under this case.

(ii) Regarding tests using formulation

Regarding the phrase "When it is found that there is no risk that the components etc. of the relevant agricultural chemical will run off into water systems such as rivers, in light of its formulation type, its application method etc.;", the following cases shall fall under this case:
(a) When the relevant agricultural chemical is used with its components enclosed, such as in the case of attractants;
(b) When the relevant agricultural chemical is used by placing it in certain locations, such as in the case of repellents, rodenticides and molluscicides;
(c) When the relevant agricultural chemical is applied to the applicable crops by painting or trunk injection;
(d) When the relevant agricultural chemical is used only inside of a facility such as warehouse and greenhouse;
(e) When the relevant agricultural chemical is not used in large quantities over a wide area at one time, such as in the case of aerosol;
(f) When the agricultural chemical is applied by attaching it directly to seeds etc., such as in the case of dust coating (excluding dipping method);
(g) When the relevant agricultural chemical is applicable for dry fields and its formulation type is granule(excluding cases where it is applied by aerial application or unmanned helicopter application), or when it is used for pricking-in hole treatment or soil injection;

② Test results regarding fish acute toxicity (additional fish species), fish(larvae) acute toxicity,

Daphnia (adult daphnids) acute immobilization, effects of coexistent organic substances on fish acute toxicity/*Daphnia spp* acute immobilization, freshwater shrimp acute toxicity, Amphipoda acute toxicity, *Chironomus* sp., acute immobilization

Regarding the phrase "When it is found that it is not necessary to conduct additional fish acute toxicity test and further tests regarding toxicity on aquatic animals and plants in conditions closer to actual environment on the basis of the test results regarding fish acute toxicity, *Daphnia spp* acute immobilization and algae growth inhibition, etc. concerning the relevant agricultural chemical;" this case shall apply if, as the result of comparison between the calculated results of acute effect concentration (AEC) and predicted environmental concentration pertaining to damage to aquatic animals and plants (short-term PEC), it is clear that a case does not fall under the cases specified under Article 3, paragraph (1), item (vi) of the Law (including cases where it is applied mutatis mutandis under Article 15-2, paragraph (6) of the Law).

③ Test results regarding *Daphnia spp* reproduction

(i) Regarding the phrase "When it is found that there is no risk that the components etc. of the relevant agricultural chemical will run off into water systems such as rivers, in light of its formulation type, its application method etc.;", the following cases shall fall under this case:

(i) When the relevant agricultural chemical is used with its components enclosed, such as in the case of attractants;

(ii) When the relevant agricultural chemical is applied to the applicable crops by painting or trunk injection;

(iii) When the relevant agricultural chemical is used only inside of a facility such as warehouse and greenhouse;

(iv) When the relevant agricultural chemical is not used in large quantities over a wide area at one time, such as in the case of aerosol;

(v) When the agricultural chemical is applied by attaching it directly to seeds etc., such as in the case of dust coating;

(ii) Regarding the phrase "When there is no risk that the relevant agricultural chemical will affect the reproduction of crustaceans because its components etc. are of very low toxicity, in light of their type etc.;", cases where the following agricultural chemicals are used shall fall under this case:

(i) Agricultural chemicals other than those that have an insect growth regulatory effect such as chitin synthesis inhibitor;

(ii) Agricultural chemicals that have an insect growth regulatory effect such as chitin synthesis inhibitor and whose estimated half-life in water is shorter than 4 days;

④ Test results regarding algae growth inhibition

(i) Regarding tests using TGAI

(i) Regarding the phrase "When it is found that there is no risk that the components etc. of the relevant agricultural chemical will run off into water systems such as rivers, in light of its formulation type, its application method etc.;", the following cases shall fall under this case:

[a] When the relevant agricultural chemical is used with its components enclosed, such as in the case of attractants;

[b] When the relevant agricultural chemical is applied to the applicable crops by painting or trunk injection;

[c] When the relevant agricultural chemical is used only inside of a facility such as warehouse and greenhouse;

[d] When the relevant agricultural chemical is not used in large quantities over a wide area at one time, such as in the case of aerosol;

[e] When the agricultural chemical is applied by attaching it directly to seeds etc., such as in the case of dust coating;

(ii) Regarding the phrase "When the relevant agricultural chemical is found not to be harmful because its components etc. are of very low toxicity, in light of their type etc.;", for example, cases where the active ingredients of the relevant agricultural chemical have already been used generally and

widely for food etc., and well known to the public as safe for aquatic plants shall fall under this case.

(ii) Regarding tests using formulation

Regarding the phrase "When it is found that there is no risk that the components etc. of the relevant agricultural chemical will run off into water systems such as rivers, in light of its formulation type, its application method etc.;", the following cases shall fall under this case:

(i) When the relevant agricultural chemical is used with its components enclosed, such as in the case of attractants;

(ii) When the relevant agricultural chemical is used by placing it in certain locations, such as in the case of repellents, rodenticides and molluscicides;

(iii) When the relevant agricultural chemical is applied to the applicable crops by painting or trunk injection;

(iv) When the relevant agricultural chemical is used only inside of a facility such as warehouse and greenhouse;

(v) When the relevant agricultural chemical is not used in large quantities over a wide area at one time, such as in the case of aerosol;

(vi) When the agricultural chemical is applied by attaching it directly to seeds etc., such as in the case of dust coating (excluding dipping method);

(vii) When the relevant agricultural chemical is applicable for dry fields and its formulation type is granule(excluding cases where it is applied by aerial application or unmanned helicopter application), or when it is used for pricking-in hole treatment or soil injection;

(4) Test results regarding toxicity on beneficial organisms other than aquatic animals and plants

Regarding the phrase "When the relevant agricultural chemical is found not to be harmful because its components etc. are of very low toxicity, in light of their type etc.;", for example, cases where the active ingredients of the relevant agricultural chemical have already been used generally and widely for food etc., and well known to the public as safe for bees, silkworms, natural enemy insects and birds shall fall under this case.

The guidelines for each test item are specified as follows:

① Test results regarding bee toxicity

Regarding the phrase "When it is found that there is no risk that bees will be exposed to the relevant agricultural chemical, in light of its formulation type, its application method etc.;", the following cases shall fall under this case:

(i) When the relevant agricultural chemical is used with its components enclosed, such as in the case of attractants;

(ii) When the relevant agricultural chemical is used by placing it in certain locations, such as in the case of repellents, rodenticides and molluscicides;

(iii) When the relevant agricultural chemical is used only inside of a facility such as warehouse where bees are not free to move;

(iv) When the relevant agricultural chemical is applied to soil (excluding insecticides and soil fumigants); when it is applied to paddy water (throw-in method, dropping method, application at irrigation inlet); when it is applied to a nursery box; when it is applied by attaching it directly to seeds etc. such as in case of dust coating; or when it is applied to the applicable crops by painting or trunk injection;

(v) When the formulation type of the relevant agricultural chemical is granule (excluding cases where it is an insecticide or where it is applied by aerial application or unmanned helicopter application);

② Test results regarding silkworm toxicity

Regarding the phrase "When it is found that there is no risk that silkworms will be exposed

to the relevant agricultural chemical through ingesting mulberry leaves etc., in light of its formulation type, its application method etc.;", the following cases shall fall under this case:

(i) When the relevant agricultural chemical is used with its components enclosed, such as in the case of attractants;

(ii) When the relevant agricultural chemical is used by placing it in certain locations, such as in the case of repellents, rodenticides and molluscicides;

(iii) When the relevant agricultural chemical is used only inside of a facility such as warehouse and greenhouse;

(iv) When the relevant agricultural chemical is applied to soil (excluding cases where its applicable crops include mulberry, or where it is an insecticide or a soil fumigant); when it is applied to paddy water (throw-in method, dropping method, application at irrigation inlet); when it is applied to a nursery box; when it is applied by attaching it directly to seeds etc. such as in case of dust coating; or when it is applied to the applicable crops by painting or trunk injection;

(v) When the formulation type of the relevant agricultural chemical is granule (excluding cases where its applicable crops include mulberry, where it is an insecticide, or where it is applied by aerial application or unmanned helicopter application)

③ Test results regarding natural enemy insect, etc. toxicity

Regarding the phrase "When it is found that there is no risk that natural enemy insects etc. will be exposed to the relevant agricultural chemical, in light of its formulation type, its application method etc.;", the following cases shall fall under this case:

(i) When the relevant agricultural chemical is used with its components enclosed, such as in the case of attractants;

(ii) When the relevant agricultural chemical is used by placing it in certain locations, such as in the case of repellents, rodenticides and molluscicides;

(iii) When the relevant agricultural chemical is used only inside of a facility such as warehouse where the habitat of natural enemy insects etc. is not found;

(iv) When the relevant agricultural chemical is applied to paddy water (throw-in method, dropping method, application at irrigation inlet); when it is applied to a nursery box; when it is applied by attaching it directly to seeds etc. such as in case of dust coating; or when it is applied to the applicable crops by painting or trunk injection;

(v) When the formulation type of the relevant agricultural chemical is granule (excluding cases where it is an insecticide or where it is applied to places other than paddy water);

④ Test results regarding avian toxicity (test results regarding avian acute oral toxicity and avian dietary toxicity)

(i) Regarding the phrase "When it is found that there is no risk that birds will be exposed to the relevant agricultural chemical, in light of its formulation type, its application method etc.;", the following cases shall fall under this case:
  (a) When the relevant agricultural chemical is used with its components enclosed, such as in the case of attractants
  (b) When the relevant agricultural chemical is applied to the applicable crops by painting or trunk injection;
  (c) When the relevant agricultural chemical is used only inside of a facility such as warehouse and greenhouse;

(ii) Regarding the phrase "As regards avian dietary toxicity tests, when it is not confirmed that the substance is highly toxic on the basis of the test results regarding avian acute oral toxicity;", cases where agricultural chemicals whose 50% lethal concentration is 300mg/kg or larger are used shall fall under this case.

(5) Test results regarding the properties, stability, degradability, etc. of active ingredients

① Regarding the phrase "When the relevant agricultural chemical is found to be safe because its components etc. are of very low toxicity, in light of their type etc.;", cases where the active ingredients of the relevant agricultural chemical have already been used generally and widely for food etc., and well known to the public as safe shall fall under this case, in principle.

② Regarding the phrase "when it is found that there is no risk that the components etc. of the relevant agricultural chemical will contaminate farmland soil where it is used or run off into water systems such as rivers, in light of its formulation type, its application method etc.;", the following cases shall fall under this case:

(i) When the relevant agricultural chemical is used with its components enclosed, such as in the case of attractants;

(ii) When the relevant agricultural chemical is used by placing it in certain locations, such as in the case of repellents, rodenticides and molluscicides;

(iii) When the relevant agricultural chemical is applied to the applicable crops by painting or trunk injection;

(iv) When the relevant agricultural chemical is used only inside of a facility such as warehouse and greenhouse;

(v) When the relevant agricultural chemical is not used in large quantities over a wide area at one time, such as in the case of aerosol;

(6) Test results regarding derivation of predicted environmental concentration

① Regarding the phrase "When it is found that there is no risk that the components etc. of the relevant agricultural chemical will contaminate farmland soil where it is used or run off into water systems such as rivers, in light of its formulation type, its application method etc.;", the following cases shall fall under this case:

(i) When the relevant agricultural chemical is used with its components enclosed, such as in the case of attractants;

(ii) When the relevant agricultural chemical is used by placing it in certain locations, such as in the case of repellents, rodenticides and molluscicides;

(iii) When the relevant agricultural chemical is applied to the applicable crops by painting or trunk injection;

(iv) When the relevant agricultural chemical is used only inside of a facility such as warehouse and greenhouse;

(v) When the relevant agricultural chemical is not used in large quantities over a wide area at one time, such as in the case of aerosol;

(vi) When the relevant agricultural chemical is applied by attaching it directly to seeds etc. such as in case of dust coating;

② Regarding the phrase "When the relevant agricultural chemical is found to be safe because its components etc. are of very low toxicity, in light of their type etc.;", cases where the active ingredients of the relevant agricultural chemical have already been used generally and widely for food etc., and well known to the public as safe shall fall under this case.

③ The term "Paddies" shall include all states where crops are cultivated in a flooding condition and include cases where they are used for the cultivation of "*kuwai* (*Sagittaria trifolia* var. *edulis*)", "lotus root", "rush", etc. besides paddy rice.

④ The phrase "When the relevant agricultural chemical is not used in paddies;" shall include

cases where it is used in paddy field before 15 days prior to the beginning of irrigation and after harvesting where there is no paddy water.

⑤ The phrase "When the relevant agricultural chemical is used only in paddies;" shall refer to the cases where it is used in paddy field only for a period from 14 days prior to the beginning of irrigation until harvesting.

⑥ The phrase "When it is found that there is no risk that the relevant agricultural chemical will drift and contaminate water systems such as rivers, in light of its formulation type, its application method etc.;" shall refer to the following cases:

(i) When the formulation type of the relevant agricultural chemical is granule;

(ii) When the agricultural chemical is directly applied to soil, such as in the case of soil incorporation treatment;

(iii) When the relevant agricultural chemical is directly applied to paddy water (throw-in method, dropping method, application at irrigation inlet etc.);

(iv) When the relevant agricultural chemical is applied to a nursery box;

(7) Test results regarding residues in crops

Test results regarding residues in crops

① Regarding the phrase "When the relevant agricultural chemical is found to be safe because there is no risk that humans will ingest its components etc. for a long period, or because the amount of ingestion of them would be extremely very small even when ingested, in light of its formulation type, its application method etc.;", for agricultural chemicals other than those referred to in both item(i)(a) and item (ii)(a) "When the relevant agricultural chemical is used for crops other than those that are to be used for food (including industrial crops and crops to be used for animal feed);" in the Appendix Table 2 in the Notification by the Director-General, the following cases shall fall under this case:

(a) When there is no risk that the applicable crops will be directly exposed to the relevant agricultural chemical, in light of its formulation type, its application method etc.; For example, the following cases shall fall under this case:

[a] When the relevant agricultural chemical is used with its components enclosed, such as in the case of attractants;

[b] When the relevant agricultural chemical is used by placing it in certain locations, such as in the case of repellents, rodenticides and molluscicides;

(b) When it is found that there is very little risk that humans will ingest the components etc. of the relevant agricultural chemical through the relevant crops because it is to be used in the early growth stage of the applicable crops such as in the case of an agricultural chemical applied by attaching it directly to seeds etc. like dust coating;

(c) Regarding test on residues in crops for thinned-out and pinched-off vegetable seedlings, when a note of caution saying "Do not use this agricultural chemical for thinned-out and pinched-off vegetable seedlings" or the like is added to the label;

② Regarding the phrase "When the relevant agricultural chemical is found to be safe because its components etc. are of very low toxicity, in light of their type etc.;", cases where the active ingredients of the relevant agricultural chemical have already been used generally and widely for food etc., and well known to the public as safe shall fall under this case.

③ The phrase "When it is found that there is no risk that the relevant spreader will affect the residue of the applicable agricultural chemicals in crops;" shall refer to the cases where it has been confirmed that the relevant spreader will not affect the residue of the applicable agricultural chemicals on the basis of the tests conducted by the following method, in principle.

Select one or more type from among crops which the spray solution easily adheres to (cucumber, napa cabbage, apple etc.) and one or more type from among crops which the spray

solution hardly adheres to (rice, wheat or the like, Welsh onion, cabbage etc.) as test crops. Select an agricultural chemical which is one of the applicable agricultural chemicals and applicable to the test crops. Then compare the difference between the residue level of the active ingredients of that applicable agricultural chemical under the presence or absence of the spreader for each test crop over time.

The number of trials and analysis for each crop shall be one, and nothing particularly is specified for the test facility.

(8) Test results regarding persistence in soil

① Test results regarding residues in soil

(i) Regarding the phrase "When it is found that there is no risk that the components etc. of the relevant agricultural chemical will contaminate farmland soil where it is used, in light of its formulation type, its application method etc.;", the following cases shall fall under this case:

(a) When the relevant agricultural chemical is used with its components enclosed, such as in the case of attractants;

(b) When the relevant agricultural chemical is used by placing it in certain locations, such as in the case of repellents, rodenticides and molluscicides;

(c) When the relevant agricultural chemical is applied to the applicable crops by painting or trunk injection;

(d) When the relevant agricultural chemical is used only inside of a facility such as warehouse where soil is not exposed;

(e) When the relevant agricultural chemical is not used in large quantities over a wide area at one time, such as in the case of aerosol;

(ii) Regarding the phrase "When the relevant agricultural chemical is found to be safe because its components etc. are of very low toxicity, in light of their type etc.;", cases where the active ingredients of the relevant agricultural chemical have already been used generally and widely for food etc., and well known to the public as safe shall fall under this case.

② Test results regarding residues in succeeding crops

Regarding the phrase "When the relevant agricultural chemical is found to be safe because there is no risk that crops cultivated after the applicable crops will be contaminated with its components etc., in light of the degree of its persistence in soil etc.;", the cases referred to in the preceding item ① and cases where the estimated half-life of the active ingredients etc. of the relevant agricultural chemical does not exceed 100 days on the basis of the test results regarding residues in soil, shall fall under this case, in principle.

Section 4 Regarding the Annex to the Notification by the Director-General "Guidelines on Preparation of Test Results Submitted When Applying for Registration of Agricultural Chemicals"

Basic matters

1. Basic concept

(1) This explanation supplements "Guidelines on Preparation of Test Results Submitted When Applying for Registration of Agricultural Chemicals" (hereinafter referred to as "the Guidelines"), and collects the facts and concepts that should be referred to when conducting tests. The Guidelines and this explanation are described for standard test methods, and accordingly they should be renewed and improved one by one according to the future development of toxicology, science and technology.

(2) The Guidelines shall not preclude persons conducting tests from having flexibility to change and improve test methods for the purpose of more accurately achieving study objectives in accordance with the characteristics of test substances etc. However, in case of changing test methods, the changed parts and the reason of the change shall be clarified and described on the test reports etc.

2. Regarding Test substances

(1) In case of using TGAI as test substance
- ① The test substance shall be equivalent to TGAI used as raw material of the sample of the relevant agricultural chemical.
- ② The test substance from the same lot shall be used during the test period, in principle. But in case that the test substance of another lot is unavoidably used, the chemical composition of ensuing lot shall be closely similar to that of previous lot.
- ③ It is necessary to clarify as much as possible, its general name, chemical name, chemical structural formula, purity, lot number, physicochemical characteristics such as vapor pressure, aqueous solubility etc. and the composition of impurities.

(2) In case of using formulations as test substance
- ① The test substance shall be equivalent to the sample of the relevant agricultural chemical.
- ② The test substance from the same lot shall be used during the test period, in principle. But in case that the test substance of another lot is unavoidably used, the chemical composition of ensuing lot shall be closely similar to that of previous lot.
- ③ It is necessary to clarify as much as possible, the name of kind, the content of active ingredients, lot number and the kinds of other components.

(3) Careful attention shall be paid to the uniformity and stability of the test substance when it is dosed to test organisms by mixing it to the feed for tests on toxicity, etc. In cases where the test substance is dosed by using a solvent, it is desirable that the toxicity of the solvent is already known and the solvent does not give serious influence to the test results.

(4) It is important to perform the health management of experimenters and the waste disposal with attention to the hazards of the test substance. Especially, sufficient attention is needed for the handling of positive control reference material that is used for mutagenicity tests etc.

3. Regarding test organisms

In order to accurately conduct safety evaluation of agricultural chemicals, it is desirable to use test organisms of the same species and strain for all the relevant test items. Refer to the corresponding items of each test with regard to the conditions of test organisms used for

individual tests.

4. Regarding handling of experimental animals

The spirit of animal welfare must not be forgotten even when conducting tests on toxicity of agricultural chemicals, because there has been a growing mood of animal welfare both at home and abroad recently. Accordingly, it is desirable to study the necessity of the experiment by collecting sufficient materials prior to the start of tests. And, in case of conducting the tests, it is also desirable to prepare cautious and sufficient planning by utilizing materials collected in advance and to conduct the experiment by using the requisite minimum animals.

<Efficacy and Phytotoxicity Test>

Test on efficacy against the applicable diseases or pests and on phytotoxicity to the applicable crops (1-1-1 to 1-1-4)

Efficacy and phytotoxicity test (1-1-1)

1. On the test method

(1) The test field must have a clarified record of spraying agricultural chemicals, and those chemicals must not have negative influence to the test results.

(2) The test methods are different from each other depending on the kind of formulations, disease, insect pest, weeds, crop plants and treatment methods, and therefore it is needed to use appropriate test methods in which the characteristics of each method is taken into consideration in order to assess the efficacy and phytotoxicity of the chemicals. And the test must be executed by the methods and chemicals concentration that will be applied for the actual registration.

Test groups should be set up, in principle in two or more replicates. Areas of the each test groups should be set up considering the kind of crop plants, diseases, insect pests, and weeds that relate to the application.

(3) Ascertain the species, form (eggs, larvae, etc.), life stage, abundance, and transition, and treatment with agricultural chemicals should be conducted at a time appropriate for the evaluation of efficacy.

Additionally, it is unavoidably acceptable to execute the tests by conducting inoculation, release or seeding depending on the proliferation of applied disease, insects and weeds. In this case, it is needed to adopt some methods that do not cause very different amount of infestation in comparison with that of natural infestation.

(4) For reference products, it is necessary to use the agricultural chemicals that are registered to the combination of applied crop plants and applied disease, insect pests and weeds that are relating to the application.

In addition, the reference products should have similar effects, and be the same type and have the same usage as the test chemicals.

(5) It is necessary to use appropriate, well-known methods by which the efficacy of chemicals and phytotoxicity can be assessed depending on the kinds of agricultural chemicals, the characteristics of objective disease, insect pests, weeds or crop plants.

2. On the items to be reported

In principle, following items shall be described in the test result report.

(1) The institution that conducted the tests and the persons in charge of tests (the department to which they belong and so on)

(2) The purpose of the tests

(3) The information on the test substance

The name of agricultural chemicals (Code name is acceptable, but clarify the relationship with the name of the agricultural chemicals at the timing of for

registration application in that case.), lot number etc.

(4) On the crop plants used for the test
① Name of agricultural products (its kind, age of tree, etc.)
② Information on the crop plants (growing status, condition of cultivation etc.)

(5) The names of objective disease, insect pests and weeds and their infestation status.
In the case of inoculation, release, or seeding of objective disease, insect pests, or weeds:
① History of disease, insects, or weeds for inoculation, release, or seeding,
② Method of inoculation, release, or seeding, etc., and
③ Amount of inoculation, release, or seeding, etc.

(6) Test site, the condition of test field (soil characteristics etc.), division and area

(7) The treatment method of agricultural chemicals (the date of treatment, growing stage of crop plants, treatment apparatus, dilution ratio, the quantity of treatment, treatment method, the number of treatments, the use of spreading agents and so forth.)

(8) Weather condition during the test (atmospheric temperature, the amount of rainfall etc.) Detailed record and consideration are needed as to such weather condition as the amount of rainfall that might have affected the test result.

(9) The use of other agricultural chemicals

(10) The method of examination (method, timing, the number of surveys etc.)

(11) Test results
① The efficacy of the agricultural chemicals observed at the test product when compared with those at the untreated control and the reference product.
② The existence of phytotoxicity of the agricultural chemicals, its situation and magnitude (the measurement of grass length etc.), the degree of recovery etc.
③ Consideration and study
④ Other items necessary to assess the test results

(12) Summary of review results by experts (if applicable)

Limit test for phytotoxicity (1-1-2)

1. Crop plants used for test
Use sound plants that are raised by conventional methods.

2. On the test method
(1) The use of a field is preferable, but the use of pots would be acceptable only if scientific assessment could be possible.

(2) The purpose of the test is to clarify the maximum amount or concentration of the agricultural chemicals that does not induce phytotoxicity, and accordingly it is preferable to increase the amount (concentration) step by step from no adverse effect level to the level that induces the phytotoxicity. However, it is acceptable to execute the

test by setting up the amount (concentration) twice as much as the maximum amount that is applied for the registration.

(3) Provide untreated control and test products with at minimum twice as large amount (concentration) as that of the maximum allowable amount (concentration) for the registration application in case of executing the test by setting up the amount (concentration) of the agricultural chemicals twice. And additionally, provide the district of the maximum amount (concentration) of agricultural chemicals in range applied for the registration depending on the necessity in order to analyze the status of occurrence of phytotoxicity.

3. On the items to be reported

In principle, following items shall be described in the test result report.

(1) The institution that conducted the tests and the persons in charge of tests (the department to which they belong and so on)

(2) The purpose of the tests

(3) Information on the test substance
The name of agricultural chemicals (Code name is acceptable, but clarify the relationship with the name of the agricultural chemicals at the timing of registration application in this case.), lot number etc.

(4) On the crop plant used for the test
① Name of crop plant (its cultivar)
② Information on the crop plants (growing status, condition of cultivation etc.)

(5) Test site, the condition of test field (soil characteristics etc.), division and area

(6) The treatment method of agricultural chemicals (the date of treatment, growing stage of crop plants, treatment apparatus, dilution ratio, the quantity of treatment, treatment method, the number of treatments, the use of spreading agents and so forth.)

(7) Weather condition during the test (atmospheric temperature, the amount of rainfall etc.) Detailed record and consideration are needed as to such weather conditions as the amount of rainfall that might have affected the test result.

(8) The use of other agricultural chemicals

(9) The method of examination (method, timing, the number of surveys etc.)

(10) Test results
① The existence of harmful effect of the agricultural chemicals, its situation and magnitude (the measurement of grass length etc.), the degree of recovery etc.
② Consideration and study
③ Other items necessary to assess the test results

Test on residual odor in tea (1-1-3)

1. On the test method

(1) The field used for the test must have a clarified record of spraying agricultural chemicals, and those chemicals should not have adverse effect to the test results.

(2) The treatment of agricultural chemicals must be done with the method applied for registration using the maximum amount (concentration) of chemicals.

(3) Provide an untreated control and the test product that is treated with chemicals on specific days prior to tea picking for the registration application. Additionally, provide a district that is treated with chemicals on the standard days prior tea picking for the test of residual odor.

(4) Gather test material (tea leaves) uniformly from the test districts and immediately make unprocessed tea in accordance with the standard method of tea manufacturing.

(5) Smell and check the residual odor of chemicals after pouring boiling water to the test material (test unprocessed tealeaves). Repeat the tests twice or more for the same test material. The test must be executed by several experienced specialists.

2. On the items to be reported

In principle, following items shall be described in the test result report.

(1) The institution that executed the tests and the persons in charge of tests (the department to which they belong and so on)

(2) The purpose of the tests

(3) The information on the test substance
The name of agricultural chemicals (Code name is acceptable, but clarify the relationship with the name of the agricultural chemicals at the timing of for registration application in this case.), lot number etc.

(4) On the crop plant used for the test
① Name of crop plant (its cultivar)
② Information on the crop plants (growing status, condition of cultivation etc.)

(5) Test site, the condition of test field (soil characteristics etc.), division and area

(6) The treatment method of agricultural chemicals (the date of treatment, growing stage of crop plants, treatment apparatus, dilution ratio, the quantity of treatment, treatment method, the number of treatments, the use of spreading agents and so forth.)

(7) Weather condition during the test (atmospheric temperature, the amount of rainfall etc.) Detailed record and consideration are needed as to such weather condition as the amount of rainfall that might have affected the test result.

(8) The use of other agricultural chemicals

(9) Information on tea manufacturing process, tea storage condition etc.

(10) The method of examination

(11) Test results
① Whether or not there is residual odor
② Consideration and study

③ Other items necessary to assess the test results

Tabaco taste test (1-1-4)

1. On the test method

(1) The test field used for the test must have a clarified record of spraying agricultural chemicals, and those chemicals must not have negative influence to the test results.

(2) The test must be done with the method applied for registration using the maximum amount (concentration) of chemicals. In principle, provide an untreated control.

(3) Pick inner and upper leaves by hand at proper harvest timing and dry them according to the drying methods depending on the cultivars of tobacco plants. Roll minutely cut leaves using thin paper to make test material piece.

(4) The check of residual odor due to agricultural chemicals shall be done by checking the smell of cut leaves and smoking taste, and this check should be executed by experienced specialists.

2. On the items to be reported

In principle, following items should be described in the test result report.

(1) The institution that conducted the tests and the persons in charge of tests (the department to which they belong and so on)

(2) The purpose of the tests

(3) Information on the test material

The name of agricultural chemicals (Code name is acceptable, but clarify the relationship with the name of the agricultural chemicals at the timing of for registration application in this case.), lot number etc.

(4) On the crop plants used for the test

① Name of crop plant (its cultivar)

② Information on the crop plants (growing status, condition of cultivation etc.)

(5) Test site, the condition of test field (soil characteristics etc.), division and area.

(6) The treatment method of agricultural chemicals (the date of treatment, growing stage of agricultural products, treatment apparatus, dilution ratio, the quantity of treatment, treatment method, the number of treatments, the use of spreading agents and so forth.)

(7) Weather condition during the test (atmospheric temperature, the amount of rainfall etc.) Detailed record and consideration are needed as to such weather condition as the amount of rainfall that might have affected the test result.

(8) The use of other agricultural chemicals

(9) Information on tobacco manufacturing process, tobacco storage condition etc.

(10) The method of examination

(11) Test results
① Existence of influence to the smoking taste
② Consideration and study
③ Other items necessary to assess the test results

Test on phytotoxicity to adjacent crops (1-2-1～3)

Test on phytotoxicity due to drift and scattering (1-2-1) and test on phytotoxicity to runoff from paddy water (1-2-2)

1. On the test method

(1) Plant pots could be used only if scientific assessment could be possible.

(2) The test must be conducted with the method applied for the registration using the maximum amount (concentration) of chemicals. Provide an untreated control.

2. On the items to be reported

In principle, following items shall be described in the test result report.

(1) The institution that conducted the tests and the persons in charge of tests (the department to which they belong and so on)

(2) The purpose of the tests

(3) Information on the test substance
The name of agricultural chemicals (Code name is acceptable, but clarify the relationship with the name of the agricultural chemicals at the timing of registration application in this case.), lot number etc.

(4) On the crop plants used for the test
① Name of crop plant (its cultivar)
② Information on the crop plants (growing status, condition of cultivation etc.)

(5) Division of districts etc.

(6) The treatment method of agricultural chemicals (the date of treatment, growing stage of agricultural products, treatment apparatus, dilution ratio, the quantity of treatment, treatment method, the number of treatments, the use of spreading agents and so forth.)

(7) Whether or no other agricultural chemicals are used

(8) The method of examination (method, timing, the number of surveys etc.)

(9) Test results
① The existence of harmful effect of the agricultural chemicals, its situation and magnitude (the measurement of grass length etc.), the degree of recovery etc.

② Consideration and study
③ Other items necessary to assess the test results

Test on phytotoxicity due to volatilization (1-2-3)

1. On the test method

(1) The test must be conducted using the method that will be applied for the actual registration. Provide an untreated control.

(2) The test crop plant and test methods differ depending on the characteristics of test substance. The following shows, as an example, a tunnel test method where cucumber seedlings are used.
① Test plant : cucumber seedlings having five or fewer leaves in five (5) pots
② The chemicals to be tested: formulation (pharmaceutical preparation)
③ Test method: Locate the pots of cucumber seedlings 50 cm apart from each other in a polyethylene semi-cylindrical tunnel of 40 cm high, 30 cm wide and 3 meter long. Put a vat containing 20 gram of test chemicals and one liter of water on an entrance of the tunnel and ventilate inside the tunnel by using a fan.
④ The duration of test: Take out the cucumber seedlings from the tunnel after continuing the above test for three days and observe them for seven days.

2. On the items to be reported

In principle, following items shall be described in the test result report.

(1) The institution that conducted the tests and the persons in charge of tests (the department to which they belong and so on)

(2) The purpose of the tests

(3) Information on the test substance
The name of agricultural chemicals (Code name is acceptable, but clarify the relationship with the name of the agricultural chemicals at the timing of registration application in this case.), lot number etc.

(4) On the crop plants used for the test
① Name of the crop plants (their cultivars)
② Information on the crop plants (growing status, condition of cultivation etc.)

(5) Division of districts etc.

(6) The treatment method of agricultural chemicals (the date of treatment, growing stage of the crop plants when treated, dilution ratio, the quantity of treatment, treatment method, etc.)

(7) Whether or not other agricultural chemicals are used.

(8) The method of examination

(9) Test results
① Whether or not there is phytotoxicity due to the agricultural chemicals, the situation and magnitude of the injury (the measurement of grass length etc.), the degree of recovery etc.

② Consideration
③ Other items necessary to assess the test results

Test on phytotoxicity to succeeding crops

Succeeding crop phytotoxicity test (1-3)

1. On the crop plants used for the test

The crop plants used for the test shall be cultivated according to conventional method.

2. On the test method

(1) Plant pots could be used only if scientific assessment could be possible.

(2) The test must be executed using the method applied for registration and the soil treated with the chemicals of the maximum amount (contamination). When the influence of the treatment is clear immediate after the use of herbicide etc. in the soil, the succeeding crops must be cultivated after the soil aging according to the conventional method.

3. On the items to be reported

In principle, following items shall be described in the test result report.

(1) The institution that conducted the tests and the persons in charge of tests (the department to which they belong and so on)

(2) The purpose of the tests

(3) Information on the test substance
The name of agricultural chemicals (Code name is acceptable, but clarify the relationship with the name of the agricultural chemicals at the timing of registration application in this case.), lot number etc.

(4) On the crop plants used for the test
① Name of crop plants (their cultivars)
② Information on the crop plants (growing status, condition of cultivation etc.)

(5) Division of districts etc.

(6) The treatment method of agricultural chemicals (the kind of soil, the date of treatment, the quantity of treatment, treatment method, aging condition of soil, the date of the treatment to the succeeding crop, growing stage of crop plants when treated, etc.)

(7) Whether or not other agricultural chemicals are used.

(8) The method of examination

(9) Test results
① Whether or not there was phytotoxicity by the agricultural chemicals, its situation and magnitude (the measurement of grass length etc.), the degree of recovery etc.
② Consideration

③ Other items that are considered to be necessary to assess the test results

<Toxicity test>

Acute oral toxicity test ～ teratogenicity test (2-1-1～18)

1. On the animals used for the test

(1) In principle, use healthful, young and mature animals whose origins are known.

(2) Make the test animals accustomed to the experiment environment prior to the start of the experiment. Usually, the test animals must be accustomed to the condition of the experiment room at least for five days before the start of experiment. (Generally, this length is a week.) After that, the test animals shall be allocated at random to test groups.

(3) When allocating test animals, careful attention shall be paid to make the range of their weight within 20% of the average weight for each sex. However, in the acute oral toxicity test, rodent animals will be between 8 and 12 weeks old at the start of the test.

(4) It is desirable that rodent animals are not older than 8 weeks and non-rodent animals are not older than 9 months at the start of the test.

(5) As to the control of test animals, environmental condition must be strictly controlled in order to obtain meaningful results. In case of raising them for a long time, careful attention must be paid to keep the environmental condition such as atmospheric temperature, humidity and air ventilation in order to avoid the occurrence of infection disease.

(6) The temperature in the breeding room shall be 22 ± 3 ℃ for rodent animals and 20 ± 3 ℃ for rabbits. The relative humidity shall be 30 to 70 %, and the lightning shall be on for 12 hours a day and off for 12 hours a day in case of using artificial lightning. Give the animals the feed generally used in laboratories and supply enough water without limitation.

1. On the method of administration

(1) Consider first the use of water in case that the test substance is solved or suspended into proper solvent. Then study the use of other solvents.

(2) If it is not possible to dose at a time, the divided dosing within 24 hours is acceptable.

(3) Prior to dosing of the test substance, it is suitable that rats are fasted overnight, and other rodents that have higher metabolic speed are fasted for shorter period. For example, no feeding for three to four hours before dosing is suitable to mice. And besides, do not feed them for three to four hours after dosing of the test substance.

2. On the period of observation

Consider elongation of the observation period when needed depending on reaction speed, the frequency of symptom's occurrences and the length of recovery time from the symptoms. In particular, observe them for longer than 14 days in case that the time from dosing to death is long. It is important to know the time from dosing to the occurrence of toxic symptom, from dosing to the disappearance of the symptom and from dosing to the death of test animals.

3. On the test procedure of fixed dose method

(1) On Annex 2-1-1-① and 2-1-1-②, the term "evident toxicity" means the sign that can expect more severe toxic symptoms or death at the next highest fixed dose.(refer to literature 1 and 2.)

(2) In cases where an animal tested at the dose level of 5 mg/kg in the sighting study dies and further confirmation of LD_{50} is needed, a maximum of four additional animals will be dosed at 5 mg/kg in addition to the animal used in the sighing study. These animals should be dosed in a sequential manner and the time interval between dosing each animal should be sufficient to establish that the previous animal dies or survives. If a second death occurs following the first death in the sighting study, the dosing sequence will be immediately terminated and no further animals will be dosed. As the results, if a second death occurs, the LD_{50} value should be "$LD_{50} \leqq$ 5mg/kg body weight" and if there is no more than 1 death, the LD_{50} value should be "5 mg/kg body weight $\leqq$ LD_{50} $\leqq$ 50 mg/kg body weight".

4. On the report

Describe in principle following items in the report.

(1) Information on the test substance

Names of test substances, their abbreviations or code numbers, chemical names, CAS numbers (when already known) and physicochemical characteristics such as purity and stability. Describe the names and the reason of selecting solvents when they are used.

(2) Information on the animals used for the tests

The species, groups, ages, sex, the supply sources, number of animals per group, individual animal's weight at the start of the test, animal raising conditions (environment, feed and water quality etc.) and so on.

(3) Information on the test conditions

Preparing method of the test substance, the reason that the dosing amount is decided, dosing method and dosing period, dosing amount etc.

(4) Test results

The kinds, degree and duration of general condition, body weight, toxicity-reaction data per sex and dosage (the number of died or slaughtered animals, and the number of animals that showed toxicity-reacted symptoms during the test period etc.), the time of death during and after dosing, the views on the results of pathological autopsies, histopathological views, the views on the results of other inspections, the methods of statistical transaction, the results of statistical transaction etc.

(5) Consideration and conclusion

LD_{50} value or the range including LD_{50} value

When all 5 animals are no toxicity in the limit test for fixed dose method, or 6 animals are no death in the limit test for toxic class method, describe it in the report.

(6) Reference literatures

5. Others

In addition to "Fixed Dose Procedure" and "Acute Toxic Class Method", as an acute oral toxicity test, there are the OECD test guidelines No. 425 "Acute Oral Toxicity – Up-and-Down-Procedure". The test may be conducted in accordance with such guidelines.

6. Literatures

(1) Van den Heuvel, M.J., Clark, D.G., Fielder, R.J., Koundakjian, P.P., Oliver, G.J.A., Pelling, D., Tomlinson, N.J. and Walker, A.P. (1990). Fd. Chem. Toxicol. 28, 469-482.
(2) OECD (2000). Environmental Health and Safety Monograph Series on Testing Assessment No 19.

Acute dermal toxicity test (2-1-2)

1. On the method of administration

Consider first the use of water in case that the test substance is solved or suspended into proper solvent. Then study the use of other solvents.

2. On the period of observation

Same as the acute oral toxicity test (2-1-1).

3. On the establishing of the number of test animals and test groups

The dosing amount to a group of another sex shall be equivalent to LD50 to confirm the sensibility.

4. Others

Any methods generally used (refer to the literature (1)-(7)) can be adopted for the calculation of LD_{50}.

5. On the report

Describe in principle following items in the report.

(1) Information on the test substance
Same as the acute oral toxicity test (2-1-1).

(2) Information on the animals used for the tests
Same as the acute oral toxicity test (2-1-1).

(3) Information on the test conditions
Same as the acute oral toxicity test (2-1-1).

(4) Test results
Same as the acute oral toxicity test (2-1-1).

(5) Consideration and conclusion
LD50 value per sex, 95% reliability limit value of LD50, dosage-death curve and inclination (when available depending on calculation methods)

(6) Reference literatures

6. Literatures

(1) Bliss, C.I., Quart. J. Pharm. Pharmacol., 11, 192-216, 1938.
(2) Litchfield, J.T. and Wilcoxon, F., J. Pharmcol. Exp. Ther. 96, 99-113,1949.
(3) Finney, D.G., Probit Analysis.(3rd Edn.)London, Cambridge University Press, 1971.
(4) Weil, C.S., Biometrics, 8, 249-263, 1952.
(5) Weil, C.S., Drug Chem. Toxicol. 6, 595-603, 1983.

(6) Thompson, W., Bact. Rev., 11, 115-141, 1947.
(7) Miller, L.C. and Tainter, M.L., Proc. Soc. Exp. Biol. Med. NY, 57, 246-264, 1944.

Acute inhalation toxicity test (2-1-3)

1. On the method of exposure

(1) In principle, the test substance is left for exposure, but the test substance is solved into or suspended with proper solvent when needed for to disperse the test substance in the inhalation equipment. And further, the test substance is occasionally smashed into smaller particles or some mediums are used for better dispersion.

(2) The test using "nose (head)-exposing method" can be conducted with relatively small amount of test substance and the absorption through non-inhalation (aspiration) routes is less (than the exposure of all body). This method is safer for test executors, too, and as the result, this method is now adopted widely.

(3) Pay sufficient attention not to make inside the exposure equipment short of oxygen. Over 12 times of ventilation per hour is needed in case of total body exposure, and more ventilation air than twice of test animals' aspiration volume is needed in case of nose-exposing method The exposure concentration shall be measured by sampling over three times from the region where the test animals inhale during the exposure. In principle, chemical analysis shall be carried out.

(4) Gather sample particles of test substance at least once from the animal-inhaling region, and calculate aerodynamic mass median diameter (MMAD) and geometrical standard deviation (GS or δ g) by conducting chemical or gravity analysis.

(5) The particle diameter that can be inhaled was formerly considered to be 10 or 15 μ m that was inhaled from nasal cavities, but recently a standard diameter has become 4 μ m that is considered to reach bronchi. It is preferable to make MMAD from 1 to 4 μ m, but it is considered to be difficult to make it smaller than 4 μ m depending on the test substance. In this case, calculate the ratio (%) of smaller particles than 4 μ m according to the result of analysis.

(6) Monitor continuously the concentration inside the inhaling equipment and decide the starting time of exposure in consideration with the time span when the concentration becomes stabilized.

(7) It is preferable that the temperature and humidity in the inhaling equipment during exposure is same as that of the raising environment (excluding the case of high concentration exposure).

2. On the period of observation

Same as the acute oral toxicity test (2-1-1).

3. On the observation and inspection

(1) On the observation of general condition, it is necessary to pay attention to the change of respiration etc. since the exposed part is respiratory tract.

(2) As to the pathological autopsies, pay attention to the respiratory organs exposed to the test substance.

4. On the report

Describe in principle following items in the report.

(1) Information on the test substance

Same as the acute oral toxicity test (2-1-1)

(2) Information on the animals used for the tests

Same as the acute oral toxicity test (2-1-1)

(3) Information on the test conditions

Preparing method of the test substance, the reason of deciding exposed amount, exposure method, exposure equipment and measurement apparatus, the measurement of concentration (calculation of measured concentration and nominal concentration), temperature and humidity measurement inside the inhaling equipment, information on the ventilation in the inhaling equipment (flow rate, the number of ventilation etc.), the distribution of particle diameters (MMAD, GS), the ratio of inhalable particles etc.

(4) Test results

Same as the acute oral toxicity test (2-1-1)

(5) Consideration and conclusion

LC_{50} values per sex, 95% reliability limit value of LC50, dosage-death curve and inclination etc. (when available depending on calculation methods)

(6) Reference literatures

Skin irritation test (2-1-4)

1. On the method of administration

(1) In case of applying test substance to skin, choose proper hair-growing cycle and avoid densely bristled part with hair.

(2) When liquid test substance cannot be used without dilution, use the diluted liquid of the highest concentration for practical use.

2. On the report

Describe in principle following items in the report.

(1) Information on the test substance

Same as the acute oral toxicity test (2-1-1)

(2) Information on the animals used for the tests

Same as the acute oral toxicity test (2-1-1)

(3) Information on the test conditions

Same as the acute oral toxicity test (2-1-1)

(4) Test results

The kinds, degree and duration of general condition, body weight, irritation data concerning individual animals and observed period, all the lesions observed, degree of observed irritation and its condition, and all other toxic action etc.

(5) Consideration and conclusion

(6) Reference literatures

Eye irritation test (2-1-5)

1. On the method of administration

(1) When liquid test substance cannot be used without dilution, use the diluted liquid of the highest concentration for practical use.

(2) When the calculation is impossible before the start of dosing, for example, in case of aerosol, the dosage shall be roughly calculated using the paper weight increase after spraying aerosol to a piece of paper whose weight is measured in advance through a hole of rabbit eye's size. For volatile substance, dosage is roughly calculated weighting the vessel before and after use. Tepid water or physiological salt solution shall be generally used for washing animals' eyes to confirm the efficacy of eye washing.

2. On the report

Describe in principle following items in the report.

(1) Information on the test substance
Same as the acute oral toxicity test (2-1-1)

(2) Information on the animals used for the tests
Same as the acute oral toxicity test (2-1-1)

(3) Information on the test conditions
Same as the acute oral toxicity test (2-1-1)

(4) Test results
Same as the skin irritation test (2-1-4)

(5) Consideration and conclusion

(6) Reference literatures

Skin sensitization test (2-1-6)

1. On the test method

Guinea pig Maximization Test (hereinafter referred to as GPM method) is recommendable for TGAI and Buehler method is recommendable for formulation.

2. On positive control group

It is recommendable to modify the background data that are alternatives of the positive control groups, to the periodically tested ones. The background data must be made anew when basic test conditions (strain, feed, housing condition, experimenter etc.) are changed in the testing facilities.

3. On GPM method

(1) Decision of the dosage

Use non-treated animals or those treated with Freund's complete adjuvant treatment (hereinafter referred to as FCA) for preliminary test.

(2) The first sensitization (by intradermal injection)

Injection 1 and 2 shall be shot to the head side of test zone adjacent to each other, and injection 3 shall be shot to the tail side. For injection 3, water-soluble substance shall be solved in aqueous phase before mixing with FCA. Fat-soluble or non-water-soluble substance shall be suspended in FCA before mixing with aqueous phase. The concentration of test substance shall be the same as that of injection 2.

(3) Observation

Chemical treatment is acceptable to remove hair after shaving, but choose the chemicals that do not induce stimulus as much as possible.

(4) Re-challenge

It is appropriate to provide a new control group for re-challenge, but when it is judged that the initial challenge concentration does not induce sensitization reaction and gives no influence to the result of re-challenge, the animals used for the initial challenge can be used for the re-challenge.

4. On Buehler method

At the preparation of test substance, use water or diluted surfactant solvent with no stimulation as solvent when the test substance is water-soluble. In case of using the surfactant with no information of sensitization, the solvent at induction can be changed at the timing of challenge. In other cases, it is preferable to use 80% ethanol water solvent for induction and acetone for the challenge.

5. On the report

Describe in principle following items in the report.

(1) Information on the test substance

Same as the acute oral toxicity test (2-1-1)

(2) Information on the animals used for the tests

Same as the acute oral toxicity test (2-1-1)

(3) Information on the test conditions

Same as the acute oral toxicity test (2-1-1)

(4) Test results

The kinds, degree and duration of general situation, body weight, sensitization data concerning individual animals and observed period, all the reactions observed, degree of observed reactions and their characteristics, and all other toxic reaction, the test results using positive control substance etc.

(5) Consideration and conclusion

(6) Reference literatures

Acute neurotoxicity test (2-1-7) and Repeated dose oral

neurotoxicity test (2-1-12)

1. On the method of administration

(1) For repeated oral dose nerve toxicity test, forceful oral dosing to test animals is acceptable in case that. (a) the test substance is unstable in the feed (drinking water), (b) the analysis of test substance contained in the feed (water) is difficult and (c) giving feed(water) to test animals should be avoided.

(2) The test substance can be dosed by being enclosed into gelatin capsules

2. On the period of administration

The dosing period is usually 90 days in case of the repeated oral dose nerve toxicity test, but when NOAEL (non adverse effect level) measured by the 90 days repeated oral dose nerve toxicity test is similar to NOAEL of general use, it becomes necessary to add one-year repeated oral dose nerve toxicity test.

3. On observation and inspection

(1) Detailed observation of condition and functional examination

① An experienced inspector (an inspector who has at least an experience to inspect and observe the situation of nerve toxicity test using positive control substance) must observe and inspect carefully, and appropriately record the results. In principle, it is preferable that a same person consistently observes and inspects a test from the beginning to the end. And besides, “blind test” shall be taken into consideration in order to avoid the influence of preconception to the test result . The blind test is a test where the observation and inspection shall be conducted without informing the inspector(s) of the content of dosed chemicals.

② Attention shall be paid to other parameters (remarks) except the dose of test substance in order not to influence the behaviors of animals. The observation shall be done in the order of less irritating items to the test animals.

③ The whole body situation and the change of behaviors shall be objectively and quantitatively studied at the detailed observation of condition. Accordingly, prior to the execution of tests, it is needed to fix judgment standard, scoring standard and standard observation process per the test institution.

④ The items of observation for detailed condition are not limited to those listed in the guiding principles. When some abnormal phenomena that are not shown in the list are observed in preparatory test and other toxicity test, those phenomena should be intensively observed.

⑤ The inspection of sensory-motional reaction in the functional examination may be conducted during the process of detailed observation of condition.

⑥ The measurement of the degree of ultromotivety (the magnitude of spontaneous movement) in the function inspection shall be conducted by dividing the period and the measuring hours so that the magnitude of movement can be assessed after the test animals are accustomed to the environment (of the measuring box). (For example, the measurement shall be done for an hour with the interval of 10 minutes from immediately after containing the animals in the measuring box.)

⑦ When nerve toxicity is suspected or acknowledged by other toxicity test, structure

activation correlation etc., the guiding principles demand that the nerve toxicity test shall include sensory-functional test, motional function test and learning-memory test that are suitable to carefully investigate the nerve toxicity. It is preferable to use measuring instruments for careful investigation, but careful consideration is needed for the actual use of instruments, because it (the use of measuring instruments) is not good in some case for the animals while they are alive. Preferably refer to the literature (1) to select test methods.

Now, it is preferable to conduct learning-memory test when histopathological-changes because of dosing test substance are observed in the area regarding learning and memory.

Remarks) The parameters to influence animals' behavior are noise level, room temperature, humidity, intensity of illumination, intensity of smell, the time of inspection, the change of environment etc.

(2) On ophthalmological inspection

Ophthalmological inspection is needed for the tests that last longer than 90 days. The inspection should be usually done on front aqueous chambers, intermediate transillumination body and eye grounds separately by using naked eyes and ophthalmoscopes. If there are results of inspection by other tests having similar test period and dosage, they (the test results) can be used as the test results.

(3) Others

It is necessary to show the reliability of inspection by using positive controlled data of test facilities concerning the detailed observation of condition, functional inspection and histopathological inspection. The background data can be used as the positive control data.

It is recommendable to modify the background data periodically (around once a year). And besides, the background data must be renewed when basic test conditions are changed in the testing facilities.

The information regarding the positive control substance can be obtained from literatures (2) ～(10).

4. On the assessment of the test results

Try to harmonize function-neurological influence and neuro-histopathological influence in order to assess the influence acknowledged by the test. And besides, take other observed toxicological influence into consideration for the assessment.

5. On the report

In principle, describe following items in the report.

(1) Information on the test substance.

Same as the acute oral toxicity test (2-1-1)

(2) Information on the animals used for the tests.

Same as the acute oral toxicity test (2-1-1)

(3) Information on the test conditions

Preparing method of test substance, the reason of deciding dosage, exposing route and the period of exposure, intake of test substance (mg/kg/day), the detailed grouping of test animals used to fix perfusion, detailed scoring methods used for the observation of detailed situation, detailed methods used for function inspection, the procedures used for inspections etc.

(4) Test results

The kinds, degree and duration of general condition, toxicity reaction data including

death per body weight, sex and dosed amount, the kinds, degree and duration of detailed situation, detailed results of functional examination, reversible or irreversible, the views on the results of pathological autopsies, histopathological and behavioral scientific views, the views on the results of other inspections, the methods and results of statistical transaction, etc.

(5) Consideration and results

The description on dosage-reaction relationship, the relationship between other toxic influence and neurotoxicity, NOAEL (no adverse effect level), the description on general nerve toxicity etc.

(6) Reference literatures

6. Literatures

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Assessment of Fore- and Hind- limb Grip Strength of Rats and Mice. Neurobehav. Toxtcol. l, 233-236.
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Acute delayed neurotoxicity test (2-1-8) and 28-day repeated administration delayed neurotoxicity test (2-1-13)

The purpose of this test is to examine whether or not there is delayed neurotoxicity of the agricultural chemicals and its no observed adverse effect level (NOAEL) by behavioral observation and biochemical & histopathological inspections.

The delayed neurotoxicity is a series of symptoms concerning delayed ataxia, distal axon hazard of spinal cord and peripheral nerves, and NTE (neuropathy target esterase)

hazard and aging in the nerve histology. Aging is formation of combined complex of NTE and phosphoryl that follows the phosphorization of active part of NTE.

1. On the animals used for the tests

Use mature hens of standard size that have evident genealogy to obtain good uniformity and repeatability. Those animals must not be infected by nerve-related disease.

2. On the method of administration

Generally, gastric tubes, gelatin capsules or something equivalent to them are used for dosing.

3. On the establishing of test groups

(1) On preliminary test

It is needed to use maximum dosage of chemicals that no death of test animals is observed. Enough number of test animals necessary for all inspections must be survived until the end of the test even when a number of animals die during the test. Accordingly, a preliminary test with proper number of test animals and dosage levels should be conducted in order to define the dosage for the main test. The lethal dosage shall be usually tried at the preliminary test.

Furthermore, it is necessary to study the maximum non-lethal dosage level when protective agents are used to avoid test animals' death due to the acute cholinergic nerve excitation. The dose of protective agent and the times of dosing shall be studied. The protective agent is occasionally used for the main test, too.

(2) On positive control groups

Positive control groups are needed for the acute delayed neurotoxicity test. The positive control groups shall be tested using neurotoxicity substance at the same time as the actual test, or recent historical data can be submitted as those of the positive control groups.

It is recommendable to update the historical data periodically (approximately once a year). And besides, new historical data must be developed when basic test conditions or test executors etc. are changed.

It is possible to use the delayed neurotoxicity substance as far as its dosage, the test animals' behavioral abnormality from it and its relation with NTE activation obstruction are examined. One of widely used substance is tri-o-clesylphosphate (TOCP).

4. On observation and inspection

(1) On the observation of general condition

The observation must include behavioral abnormality, gait ataxia and paralysis, but it shall not restricted to these items and all the toxic symptoms must be carefully observed and recorded. The observation inside breeding cages is not sufficient since it is important to confirm the occurrence of nerve symptoms for the delayed neurotoxicity test. Accordingly, it is necessary to check precisely the hens' behavior by having them forcefully do such motions as ladder climbing after letting them out from the cages at least twice a week.

The ataxia must be assessed in accordance with the assessment standard composing of four or more steps, and accordingly the testing institutes must prepare a assessment standard prior to the execution of the test. Literature (1) can be referred to for preparing the standard of assessment.

(2) On the biochemical test

NTE activity means esterase activity which is one of the enzymatic activities that hydrolyze the phenylvalarate of substrate. The esterase activity is not inhibited by

paraoxon that does not induce delayed neurotoxicity, but it is inhibited by mipafox that induces delayed neurotoxicity.
Any method using TOCP can be used to measure the NTE activity if there are some proper methods. Refer to literatures (2) – (5) for this item.

When determining the sampling time to prepare brain and lumbar spinal cord, it should be judged by observing the onset of cholinergic symptoms to know whether or not the test substance disappears slowly.

The parts of sampling are brain and lumbar spinal cord, but it is useful to include sciatic nerve. It sometimes helps the assessment to measure acetylcholine esterase (ACh E) activity, but it is needed to pay attention to the fact that the assessment of ACh E obstruction action of test substance may be minimized since the ACh E activity sometimes spontaneously reactivate itself at in-vitro tests.

5. On the assessment of the test results

Concerning the findings obtained by the tests, assess occurrence ratio, degree and their mutual relationship of behavioral, biochemical and histopathological changes and also other changes that are observed independently at the test group and the control group.

Incidentally, not all the organophosphorus compounds that obstruct NTE activity induce delayed neurotoxicity, but most of the organophosphorus compounds that induce the delayed nerurotoxocity obstruct NTE activity.

(Exception: 2,4,5-trichlorophenyl ether phosphonotioate does not restrain NTE activity but induces delayed neurotoxicity.)

6. On the report

In principle, describe following items in the report.

(1) Information on the test substance.
Same as the acute oral toxicity test (2-1-1)

(2) Information on the animals used for the tests.
Same as the acute oral toxicity test (2-1-1)

(3) Information on the test conditions
preparing method of test substance, the reason of deciding dose, exposing route and the period of exposure, intake of test substance (mg/kg/day) etc.

(4) Test results
the kinds, degree and duration of general condition, body weight, toxicity reaction data including death per dose, reversible or irreversible, the methods and findings of the biochemical inspection, necropsy findings, histopathological findings, the findings on the results of other inspections, the methods and results of statistical processing, etc.

(5) Consideration and results

(6) Reference literatures

7. Literatures

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90-day repeated dose oral toxicity test (2-1-9)

1. On the method of administration

(1) Gavage administration is acceptable in case that the test substance is unstable in the feed (drinking water), the analysis of test substance mixed in the feed (water) is difficult and the feeding (water supplying) shall be avoided.

(2) The test substance can be dosed by enclosing into gelatin capsules

(3) Use the same dosing method in the same manner as long term test, when the study is used as dose range-finding study for long term test.

2. On the period of administration

The number of dosing days is in principle 7 days per week. Five days per week are also acceptable in case of gavage administration.

3. On the number of test animals and establishing of test groups

Satellite groups may be provided to observe the recovery condition etc. from toxic symptoms. The number of animals used for satellite groups shall be 5 or more for both male and female rodents and 4 for both male and female non-rodent animals. All animals shall be raised for longer than 28 days after the end of dosing.

4. On observation and inspection

(1) Refer to the neurotoxicity test as to the observation of detailed condition and functional examination.

(2) When the test substance is dosed by being mixed in the feed (or water in case of water dosing), the intake shall be calculated by measuring the consumed amount of feed (or water). The consumed amount of feed (or water) may be measured on the basis of groups or individual animals in case of rodent animals. Measure the amount of consumed feed (water) before the start of dosing depending on the necessity.

(3) The urine examination of rodents can be conducted on individual animal basis or on the male and female groups basis.

(4) Ophthalmological inspection shall be usually conducted for front aqueous chambers, optic media and eye grounds separately by naked eyes and ophthalmoscopes.

(5) When the influence to genital organs is considerable, it is helpful for the assessment to conduct the histopathological inspection of all organs of animals belonging to the dose groups except the high dose groups.

(6) The items to influence nervous system are specific nervous symptoms concerning the observation of detailed condition and functional examination and such histopathological changes as neuron degeneration and axonal degeneration of brain, sciatic nerve and spinal cord.

(7) The items to influence immune system are thymus atrophy, the weight change of organs such as the increase of spleen weight and the abnormality of immune tissues such as the histopathological change like the hypertrophy of lymphatic organs and the increase of lymphocytes.

(8) When some influence to immune system is observed, generally the immune inspection shall be conducted after the repeated dosing of test substance for a month. Refer to the literature of below when actual test is conducted.

(9) The items to influence endocrine system are weight change of endocrine organs such as adrenal gland, testis, ovary, thyroid gland, parathyroid gland, hypophysis etc. and histopathological changes such as atrophy, degeneration, necrosis and hypertrophy.

5. On the analysis of the test result

Show the obtained results by tables or figures and add the consideration to them. Prepare an overall table to summarize the results of all groups, and also prepare tables to show the data of individual animals depending groups. These tables must be referable whenever necessary.

The test results shall be analyzed using a proper statistical method that is selected at the timing of planning tests.

6. On the report

In principle, describe following items in the report.

(1) Information on the test substance.
Same as the acute oral toxicity test (2-1-1)

(2) Information on the animals used for the tests.
Same as the acute oral toxicity test (2-1-1)

(3) Information on the test conditions
preparing method of test substance, the reason of deciding the dosage, administration method and the period of exposure, intake of test substance (mg/kg/day) etc.

(4) Test results
the kinds, degree and duration of general condition, body weight, intake of feed (water), toxicity reaction data including death per dosage and sex, the kinds, degree and duration of the detailed condition, result detail of functional examination, reversibility or irreversibility, blood inspection, blood biochemical inspection, urine examination, ophtalmological inspection, necropsy findings, weight of organs, histopathological findings, the findings on the results of other inspections, the methods and results of statistical processing, etc.

(5) Consideration and results

(6) Reference literatures

7. Literature

(1) US EPA OPPTS(1998) Health Effect Test Guideline,OPPTS870.7800 Immunotoxicity

21-day repeated dermal toxicity test (2-1-10)

1. On the period of administration

Dosing days per week is in principle 7 days, but 5 days a week is acceptable when unavoidable.

2. On the number of test animals and establishing of test groups

Satellite groups may be provided to observe the recovery condition etc. from toxic symptoms. The number of animals used for satellite groups shall be 5 or more for both male and female animals, and they shall be raised for longer than 14 says after the end of dosing.

3. On the analysis of the test results

Show the obtained results by tables or figures and add the consideration to them. Prepare an overall table to summarize the results of all groups, and also prepare tables to show the data of individual animals depending groups. These tables must be referable whenever necessary.

The test results shall be analyzed using a proper statistical method that is selected at the timing of planning tests.

4. On the report

In principle, describe following items in the report.

(1) Information on the test substance.
Same as the acute oral toxicity test (2-1-1)

(2) Information on the animals used for the tests.
Same as the acute oral toxicity test (2-1-1)

(3) Information on the test conditions
Same as the 90 days repeated oral dose toxicity test

(4) Test results

the kinds, degree and duration of general condition , body weight, intake of feed, toxicity reaction data including death per dose and sex, reversible or irreversible, blood inspection, blood biochemical inspection, urine examination, necropsy findings, weight of organs, histopathological findings, the findings on the results of other inspections, the methods and results of statistical processing, etc.

(5) Consideration and results

(6) Reference literatures

90-day repeated inhalation toxicity test (2-1-11)

1. On the method of exposure

(1) Use the inhalation equipment of whole body exposure type or nose exposure type.

(2) Keep the average exposure concentration measured for 3 months inside the inhalation equipment within 20 % fluctuation of set value.

(3) The detailed information shall be referred to the acute inhalation toxicity test.

2. On the number of animals and establishing of test groups

Satellite groups may be provided to observe the recovering condition of toxicity symptoms. The size of the satellite groups should be ten or more for both male and female groups, and they must be raised for over 28 days after the end of dosing.

3. On observation and inspection

(1) It is necessary in case of the observation of general situation to pay special attention to the change of respiration etc. since the exposed part is respiratory tract.

(2) As to pathological necropsy, pay attention to the respiratory organs exposed to the test substance.

4. On the analysis of the test results

Show the obtained results by tables or figures and add the consideration to them. Prepare an overall table to summarize the results of all groups, and also prepare separate tables to show the data of individual animals depending groups. These tables must be referable whenever necessary.

The test results shall be analyzed using a proper statistical method that is selected at the timing of planning tests.

5. On the report

In principle, describe following items in the report.

(1) Information on the test substance.
Same as the acute oral toxicity test (2-1-1)

(2) Information on the animals used for the tests.
Same as the acute oral toxicity test (2-1-1)

(3) Information on the test conditions
Same as the acute inhalation toxicity test

(4) Test results

the kinds, degree and duration of general condition, body weight, intake of feed, toxicity reaction data including death per dosage and sex, reversible or irreversible, blood inspection, blood biochemical inspection, urine examination, ophthalmological inspection, necropsy findings, weight of organs, histopathological findings, the findings on the results of other inspections, the methods and results of statistical processing, etc.

(5) Consideration and results

(6)Reference literatures

1-year repeated dose oral toxicity test (2-1-14)

This test is needed to clarify the chronic effect of agricultural chemicals to mammals and to find the dose-reaction relationship by repeatedly dosing for long time.
It is necessary to make the detection of general toxicity by the execution of this test and to

obtain NOAEL (no observed adverse effect level) of the chemicals by knowing the neurological, physiological, biochemical, blood scientific points of views

1. On the method of administration

(1) Forceful oral dosing to test animals is acceptable in case that the test substance is unstable in the feed (drinking water), the analysis of test substance contained in the feed (water) is difficult and the feeding (water supplying) shall be avoided.

(2) The test substance can be dosed by being contained into gelatin capsules.

(3) Use the same dosing method as that for carcinogenic test when this test is conducted as a preparatory test for carcinogenic tests.

2. On the period of administration

The dosing period is in principle seven days a week, and it shall be continued over a year (52 weeks). However, five days per week is generally acceptable in case of forceful oral dosing.

5. On observation and inspection

(1) The intake measurement of feed (water) should be done on the basis of individual animals or groups in case of rodent animals. The measurement before the start of tests shall be done when necessary.

(2) The urine examination of rodent animals can be conducted on the basis of individual animals or male/female groups.

(3) Ophthalmological inspection shall be usually conducted for front aqueous chambers, optic media and eye grounds separately by naked eyes and ophthalmoscopes.

(4) In addition to the items shown in the guiding principles for the pathological inspection, it will be used as reference to measure the weight of as many organs as possible including lung (of both rodent and non-rodent animals) that is an important organ, and other target organs at the 90 days repeated oral dose toxicity test. It is also useful to assess the influence on endocrine tissues to measure the rats' thyroid gland / parathyroid, pituitary gland, uterus, prostate (ventral), seminal vesicle/coagulation gland.

6. On the report

The report must include complete and exact description of test procedures and all the information necessary to assess the test results. It (the report) must also include the summary of data, data analysis and the conclusion extracted from the analysis. It is required to describe in the summary the data or observed items and the difference from the control data that indicate toxic action.

In principle, following items must be described in the report.

(1) Information on the test substance
Same as that of the acute oral toxicity test

(2) Information on the animals used for the test
Same as that of the acute oral toxicity test

(3) Information on the test conditions
Same as that of the 90 days repeated oral dose toxicity test

(4) Test results

the kinds, degree and duration of general situation, body weight, intake of feed (water), toxicity reaction data including death per dosage and sex, reversibility or irreversibility, blood inspection, blood biochemical inspection, urine examination, ophthalmological inspection, weight of organs, autopsy findings, histopathological views, the views on the results of other inspections, the methods and results of statistical transaction, etc.

(5) Consideration and conclusion

Dose-reaction relation, NOAEL (no observed adverse effect level)

(6) Reference literatures

Carcinogenicity test (2-1-15)

The purpose of this test is to clarify the development of tumor-related lesions due to the intake of object agricultural chemicals by dosing various amounts of the chemicals to the test animals during most of their life spans.

1. On the method of administration

(1) Forceful oral dosing to test animals is acceptable in case that the test substance is unstable in the feed (drinking water), the analysis of test substance contained in the feed (water) is difficult and supplying feed (water) shall be avoided.

(2) The test substance can be dosed by being contained into gelatin capsules.

2. On the period of administration

The dosing period must have proper time span to achieve the test purpose taking the average longevity of the species and group of test animals into full consideration.

In general, the dosing period is 24 months (104 weeks) or more for rats and 18 months (78 weeks) or more for mice, but it must not exceed 30 months (130 weeks) for rats and 24 months (104 weeks) for mice respectively. The test substance must be dosed in principle seven (7) days a week, but five (5) days a week is acceptable for the case of forceful oral dosing.

3. On the establishing of test groups

(1) At least three-step dosing groups are needed for the purpose of checking the dosage–reaction relationship regarding carcinogenic substance. However, special careful attention shall be paid to decide the dosage since it (the setting of dosage) is very important parameter to affect the test results. Now, the dosage is not necessarily same for both male and female groups.

(2) The maximum dosage is the amount by which the death rate does not increase significantly in comparison with control groups due to the causes excluding tumor but some toxic effect is observed. But careful attention shall be paid to setting the dosage because the animals are dosed for most of their life spans. As the result, it becomes impossible to assess the carcinogenicity if the amount is too little, and it is also impossible to assess the carcinogenicity, either, if too many animals die because of too large dosage. Accordingly, it is very important to carefully consider the result of preparatory tests that were carried out for the purpose of predicting the maximum dosage.

(3) Furthermore, sufficient attention shall be paid to the increasing steps of dosage. When too many animals die when the maximum dosage is dosed, the second maximum dosage amount can be used as the maximum dosage as far as it does not differ much from the maximum dosage. But if this difference is too much, the carcinogenicity may be overlooked because it (the second largest dosage) is too little to assess the carcinogenicity. For this reason, it is requested to set all three steps with relatively large amount of dosage, and this is what is meant by “In general, the minimum dosage must not be less than 10 % of the maximum amount.”

4. On observation and inspection

(1) Pay sufficient attention to the health condition of animals, and quickly conduct pathological autopsy whenever dead animals are found. Furthermore, continue to observe the animals so that weakened animals can be disposed with appropriate

treatment such as isolation, slaughter or pathological autopsy as quickly as possible. Be careful that the test itself may possibly become invalid if the examples of invalid histopathological examination exceed 10 % of all due to cannibalism, self-fusion etc.

(2) Pay special attention to the development of tumors, and record the time of tumor development, the position of development, outward appearance (size etc.), progress etc.

(3) Measure the consumed feed (water) of individual animals or their group during the dosing and calculate the intake of test substance by using the measurement. Measure the consumed feed (water) before the dosing of the chemicals when needed.

(4) The measurement of organs' weight in the pathological examination can be referred to for the assessment

5. On the assessment of the test results

(1) Investigate the frequency of development, reaction to the dosage and the period of development regarding tumor lesions including hyperplasia and precancerous lesion on the basis of obtained test results.

(2) There are following WHO standard of judgment (see the literature (1)) as the positive judgment of carcinogenic tests. Assess the test results by referring to this standard.
①The case that the development of the different-type tumor that is not found in the control groups is observed.
②The case that the tumors observed in the control groups are observed in the dosed group with higher frequency.
③The case that tumors are observed in more kinds of organs and tissues than the control groups.
④The case that the control and dosed groups have no difference with each other in cancer incidence but the development of tumors in the dosed groups is observed earlier than those in control groups.

6. Others

The carcinogenic tests are conducted using experiment animals, but its final purpose is to judge the dangerousness or the safety of the test substance to human bodies. The tests using experiment animals are executed based on the fact that most carcinogenic substance to human bodies shows the same characteristics to experiment animals. And therefore, we should have the standpoint that the carcinogenic substance to test animals shows similar actions to human bodies unless there are clear scientific reasons against this standpoint.

However, it is overhasty to interpret that the test substance is dangerously carcinogenic to human bodies only by the fact that it (the test substance) generated tumors to the test animals at the carcinogenic tests. In order to assess the dangerousness of the test substance to human bodies, it is needed to assess the carcinogenicity with the consideration of many important parameters (for example, mutagenicity, the mechanism by which carcinogenicity works, target organs of carcinogenesis etc.) by fully utilizing all the data obtained until present.

And accordingly, when the carcinogenicity assumedly due to non-genetic toxicity is observed at the carcinogenic tests, it is needed to investigate assumed mechanism by additional tests etc. and to examine NOAEL about carcinogenicity using proper parameters. Following steps are generally and usually used to carry out this investigation.

Firstly, it is necessary to consider the mechanism of the test substance to animals. It is needed to clarify whether the test substance or metabolite is so-called genetic toxic carcinogenic substance that harms genes of the target cells or non-genetic toxic

carcinogenic substance that does not directly harm the genes. For this purpose, it shall be carried out, depending on the necessity, to investigate irregular DNA synthesis, DNA injury and adduct formation, to search cells multiplication activity like DNA synthesis and promoter action using two-stepped carcinogenic model and to investigate the influence to endocrine circulation.

Secondly, various additional tests shall be carried out to predict to what extent the actions generated by assumed mechanism occur on human bodies.

And besides, since a threshold can be set for non-genetic carcinogenic substance, it is needed to investigate NOAEL by carrying out long-term tests with smaller dosage and/or middle-term carcinogenic tests utilizing appropriate organic barometers (for example, DNA synthesis, precancerous lesion etc.) It is also important to search the activation route of the test substance and to investigate the difference among species as to metabolic activation and detoxification mechanism. Collecting information on whether or not the metabolic activation occurs specially strong is also very important.

7. On the report

The report must include complete and exact description of test procedures and all the information necessary to assess the test results. It (the report) must also include the summary of data, data analysis and the conclusion extracted from the analysis. It is required to describe in the summary the data or observed items and the difference from the control data that indicate toxic action.

In principle, following items shall be described in the report.

(1) Information on the test substance
Same as that of the acute oral toxicity test

(2) Information on the animals used for the test
Same as that of the acute oral toxicity test

(3) Information on the test conditions
Same as that of the 90 days repeated oral dose toxicity test

(4) Test results
the kinds, degree and duration of general situation, body weight, intake of feed (water), toxicity reaction data including death per dosed amount and sex, the generated place, period and appearance (size etc.) of tumor, hematologic test, autopsy findings, histopathological views, detailed description of tumor lesions with background data, the views on the results of other inspections, the methods and results of statistical transaction, etc.

(5) Consideration and conclusion
Dosage-reaction relation, NOAEL (no observed adverse effect level)

(6) Reference literatures

8. Literatures

(1) WHO: Technical Report Series No.426 (1969)

1-year repeated dose oral toxicity / carcinogenicity combined test (2-1-16)

This is basically same as the one year repeated oral dose toxicity test and carcinogenicity test.

Reproductive toxicity test (2-1-17)

1. On the animals used for the test

Other animals than rats can be used when there is proper reason such as that they have higher sensitivity to the test substance than rats.

2. On the period of administration

The time of the slaughters for P and F1 shall be decided after confirming that the re-crossbreeding is not needed.

3. On the crossbreeding, adjustment of littermate numbers and selection of the second generation (F1)

Slaughtered littermates shall be used for pathological autopsy.

4. On the observation and inspection

(1) Confirm the life and death of animals at least twice a day and besides observe the detailed situations once a week.

(2) As the parameters to observe sexual maturation, the separation of foreskin is used for male and the age-in-day of vaginal opening is used for female animals. When the influence of the dosed test substance to the sexual maturation is observed, conduct proper inspection of F2 offspring such as measuring the length between anus and the projection of reproductive organ.

(3) The observation of estrus cycle shall be continued until copulations are confirmed after the start of crossbreeding. The estrus cycle shall be measured at the last slaughter since the uterus weight fluctuates significantly depending on the step of estrus cycle. Attention must be paid not to induce pseudopregnancy when extracting smegma.

(4) Each one of testicle and epididymis should be used for spermatozoa inspection and other testicles and epididymis shall be fixed for pathological inspection. Observe the situation of over 200 sperms in the epididymis, divide the sperms into normal and abnormal ones and calculate the ratio of normal or abnormal ones.

(5) Use Bouin fluid or other proper fixing liquid that is suitable for keeping the structure of seminiferous tubule to inspect testicles.

(6) As to ovaries, it is preferable to observe the existence of luteal bodies and growing oocytes by preparing cut pieces in order to make statistical assessment. As to the second generation (F1), it is preferable to measure the numbers of mature and immature oocytes.

5. On the analysis of test results

Show the obtained results by tables or figures and add the consideration to them. Prepare a overall table to summarize the results of all groups, and also prepare separate tables to show the data of individual animals depending on individual groups. These tables

must be referable whenever necessary.

With regard to the statistical analysis, it is preferable not to regard the offspring before weaning period as independent specimens but to regard a littermate as a specimen unit. Consideration must include NOAEL to the reproduction of parent animals and the growth of the offspring at the object tests.

6. On the report

Following items shall be described in the report in principle.

(1) Information on the test substance

Same as the acute oral toxicity test

(2) Information on the animals used for the test

Same as the acute oral toxicity test

(3) Information on the test condition

preparing method of test substance, the reason to decide the dosage, dosing method and period, dosage, crossbreeding method, the method to adjust the number of raised offspring, the intake of the test substance (mg/kg/day) etc.

(4) Test results

① The influence to parent animals (general situation, body weight, intake of feed, intake of test substance, sexual maturation, estrus cycle, pregnancy, birth and growing situation, the result of sperm inspection, views on the pathological autopsies, weight of organs, views on the histopathological inspection and other inspections, the method and results of statistical transaction etc.)

② The influence to the offspring (general situation, the existence of external skin abnormality, number of born offspring, the ratio of sex, the number of survived offspring, body weight, views on the pathological autopsies, weight of organs, views on the histopathological inspection and other inspections, the method and results of statistical transaction etc.)

(5) Consideration and conclusion

Dosage-reaction, NOAEL (no observed adverse effect level)

(6) Reference literatures

Teratogenicity test (2-1-18)

1. On the animals used for the test

Pregnant female animals shall be raised separately and individually.

2. On the method of administration

Dose the test substance every day and approximately at the same time of the day.

3. On the period of administration

Start to calculate the days of pregnancy from the day of artificial fertilization as the day of zero when rabbits are artificially fertilized.

4. On the establishing of the number of animals and test groups

The number of pregnant animals per group is generally 20 or more for rats and 16 or

more for rabbits.

5. On the observation and inspection

(1) General situation of mother animals shall be observed at least twice during dosing before end after the dosing of the test substance. Record the duration etc. when some influence of dosing the test substance is observed.

(2) Ammonium sulfide etc. is used to dye uterus for the examination of implantation mark.

(3) The inspection of bone abnormality shall be preferably carried out for the bones and cartilage in case of rats.

(4) When macroscopic change is observed at placentas, measure the placental weight of individual surviving fetuses.

6. On the analysis of the test results

Show the obtained results by tables or figures and add the consideration to them. Prepare an overall table to summarize the results of all groups, and also prepare separate tables to show the data of individual animals belonging to the groups. These tables must be referable whenever necessary.

At the statistical analysis, it is preferable to make a littermate as a specimen unit.

Consideration must include the views on NOAEL to the parent animals and the fetus according to the object tests.

7. On the report

Following items shall be described in the report in principle.

(1) Information on the test substance
Same as the acute oral toxicity test

(2) Information on the animals used for the test
Same as the acute oral toxicity test

(3) Information on the test condition
Preparing method of test substance, the reason to decide the dosage, dosing method and period, dosage, crossbreeding method etc.

(4) Test results
① The influence to parent animals (general situation and pregnant situation, body weight, intake of test substance, views on the pathological autopsies, views on other inspections, the method and results of statistical transaction etc.)
② The influence to the offspring (number of dead embryo and fetus, number of survived fetus, the ratio of sex, body weight, results of the teratological tests on outer surface, internal organs and bones, views on other inspections, the method and results of statistical transaction etc.)

(5) Consideration and conclusion
Amount –reaction, NOAEL

(6) Reference literatures

Mutagenicity test (2-1-19-1～3)

On the selection of test method

The following shows examples of additional tests that shall be carried out when positive reaction is observed or suspected by "reverse mutation test using bacilli", "chromosomal abnormality test using cultured mammal cells" or "the micronucleus test using rodents" or if additional tests are required for other reasons. The other tests can be used if it is possible to appropriately assess the mutagenicity of the test substance by using those tests.

1. The tests whose barometer is gene mutation
   (1) Gene mutation test using cultured mammal cells.
   (2) Gene mutation test using drosophilae
   (3) Gene mutation test using rodent animals (including trans-genic animals)

2. The tests whose barometer is chromosomal abnormality
   (1) Chromosomal abnormality test using bone marrow cells of rodent animals
   (2) Chromosomal abnormality test using reproductive cells of rodent animals
   (3) Eugenic lethality test using rodent animals

3. The tests whose barometer is DNA injury
   (1) DNA recovery test using bacilli (Rec-assay etc.)
   (2) Umu test using bacilli
   (3) Irregular DNA synthesis (UDS) test using mammal cells
   (4) Sister chromatid exchange (SCE) test using mammal cells
   (5) Comet assay using mammal cells

Reverse mutation test (2-1-19-1)

1. On the strains used for the test

The chemical structure etc. shall be taken into consideration in order to select the strains used for the test. For example, select colon bacilli WP2 or WP2/pKM101, or rat typhoid bacilli TA102 that have remove-recovery capability instead of colon bacilli WP2uvrA for the test substance (for example –Mitomycin-C) that forms bridges among DNA chains.

Obtain quality-guaranteed strains from appropriate research institutes and periodically confirm the characteristics of test strains (about amino acid requirement, whether or not there is drug-resistance factor, sensitivity or membrane variation to ultraviolet rays etc.)

2. On the steps of dosage

In case that mutagenicity is observed at the dosage setting test, select proper dosage intervals (usually geometric ratio of 2 or 3) in which dose-dependency is required at the final test.

Record the fact in such cases as that the growth inhibition of bacilli is observed or the test substance is precipitated on the plate. The barometers of growth inhibition are the decrease of reverse variant colonies, making background lawn transparent, or survival ratio of bacilli.

3. On the control

In principle, add solvent only to negative control. When the test substance is water-solvent, it shall be solved into sterilized water. Non water-solvent test substance is usually used by being solved or suspended in DMSO. In case that water and DMSO are not appropriate as solvent, select proper solvent taking solubility and stability of the test substance and the toxicity to test strain and S9Mix into consideration. When the solvent without background data is used, add non-treated control. Use appropriate, well-known mutagenic substance for positive control depending on the kinds of test strains and existence of metabolic activity.

4. On the number of used plates

Using three or more plates is recommended according to the overseas guide-lines.

5. On the test method

The S9 concentration in S9mix is usually 10 %. However, there are other cases that lower S9 concentration is the optimum (for example aromatic hydrocarbons) and higher S9 concentration is the optimum (for example nitrosamine). And therefore, select the most suitable S9 concentration for the chemical structure of the test substance.

And furthermore, the S9 of other animals than rats must be used depending on the chemical structure of the test substance. (for example, the mutagenicity of phenacetin and bucetin is detected by the S9 of hamsters. Accordingly, select the S9 extracted from suitable species of animals considering the chemical structure of the test substance.

6. On the observation

The number of reverse variant colonies shall be counted by naked eyes or by using automatic colony counter, but use naked eyes when the use of automatic colony counter is impossible due to the deposition of the test substance on the plate. Observe the growth inhibition situation of bacilli by using a stereoscopic microscope and confirm the existence of anti-bacteria action.

7. On the judgment of the test result

The clear increase of the reverse variant colonies number means as a standard that the number of reverse variant colonies per plate increased more than twice as many at least for a strain. Statistical method shall be used as an auxiliary measure for objective assessment, and the final judgment shall be made collectively after the full consideration of biological meaning.

8. On the report

Show by tables the measured values of the number of reverse variant colonies per plate and the average value per group, and show by figures the relation between the number of reverse variant colonies and dosage.

The following must be described in the report.

(1) Information on the test substance
Same as the acute oral toxicity test.

(2) Information on the strain used for the test
Kinds of test strain, route of acquisition, etc.

(3) Information on the test condition
The reason to decide the dosage, composition of culture medium, negative and positive control, preparing and treatment method of S9 and S9mix, operational procedure of experiment, number of plates, judgment standard of the test result etc.

(4) Test result

Measured values and average value of reverse variant colonies' number, correlation diagram between reverse variant colonies' number and dosage, existence of growth inhibitors, whether or not there is deposition of test substance, background data etc.

(5) Consideration and conclusion

(6) Reference literatures

Chromosomal aberration test (2-1-19-2)

1. On the cells used for the test

Use the cells whose appearance ratio of chromosomal aberration at non-treatment groups is as low as possible for this test. Culturing cell for a number of generation during long time may possibly cause the change of cell characteristics such as the increase of natural appearance ratio of chromosomal aberration. Accordingly, when some change is observed on the cell characteristics, pick up new cells that are stored by freezing.

2. On the steps of dosage

(1) Examine cell multiplication inhibition ratio of single layer cultured cells by measuring cell density or cell number on the plate. Examine cell multiplication inhibition ratio of suspension cultured cells by measuring cell number or using mitotic index. For both cases, obtain the information on the groups using metabolic activity and the groups that do not use it.

(2) When cell toxicity is acknowledged in the precipitation dosage region, include two or more steps of the dosage that show precipitation in the final tests.

(3) When it is considered that the test was conducted under non-physiological condition such as significant cell toxicity or the change in the pH value and osmotic pressure of culture solution, submit the data concerning the condition and the results.

3. On the control

In principle add solvent only for the negative control. If the test substance is water-soluble, solve it to sterilized physiological saline solution etc. Non-water-soluble substance shall usually be solved in DMSO solvent. If it cannot be solved in DMSO, it shall be suspended in CMC solvent. If it is not suitable to solve the test substance in both water and DMSO, select a proper solvent with consideration of solubility and stability of the test substance and its toxicity to test strain and S9Mix.

Add non-treatment control in case of using a solvent without background data. For positive control, use proper known chromosomal aberration inducing substance depending on the existence of metabolic activity.

4. On the number of plates used for the test

A plate can be used in case that there are some background data showing only a little deviation of aberration ratio between two plates.

5. On the test method

The cell cycle of Chinese hamster's strain cells is generally 12 ～17 hours and accordingly its 1.5 cell cycle is equivalent to 18 ～26 hours. Decide the time of making specimens according to the cell cycles of individual cell strains.

It is known that the cell cycles are significantly delayed by the substances that inhibit DNA synthesis and block cell cycles. The numerical chromosomal aberration is observed at the metaphase of the second cell division, confirm it by carrying out longer continuous treatment, for example 48 hours continuous treatment. In case that it is needed to carry out confirmation tests for the groups using metabolic activity, it shall be taken into consideration to make specimens at slower cycle than 1.5 cell cycles and to change the amount of S9.

6. On the observation

(1) Suppose that the gaps are achromatic positions that are narrower than the chromatid width, and are existed along the chromatid axis. The structure abnormality shall be classified to chromatid type and chromosomal type, and both types shall be classified to cutting type and exchange type respectively. Record the number of all types.

(2) When many complicated aberrations per cell are observed, do not necessarily record the number of aberrations, but record the abnormal cells separately. But the number of these types cells shall be included in the total number of cells having structure abnormality.

(3) There are aneuploidy and polyploidy as the abnormality of chromosomal numbers. It is difficult to judge the induction of aneuploidy as to established cells, record polyploidy number only (including endoreduplication).

7. On the judgment of test results

(1) Divide the total aberrations to structural aberration and numerical aberration, and make judgment for both cases.
   ① As to the structural aberration, make judgment by using the total number of abnormal cells excluding gaps.
   ② As to the numerical aberration, the total number of polyploidy and endoreduplicated types shall be used for the judgment and make separate judgment for both types when necessary.

(2) Statistical method shall be used as an auxiliary method for objective assessment and the final judgment shall be collectively made taking biological meaning into full consideration.

8. On the report

To show the total number of abnormal cells, use the total number of abnormal cells excluding those having only gap aberration. Show the abnormal cells of gap only separately.

Following items shall be in principle shown in the report.

(1) Information on the test substance
   Same as the acute oral toxicity test

(2) Information on the cells used for the test
   Kinds of cells, acquisition route of the cells, kinds of culture solution, lot number of blood serums etc.

(3) Information on the test condition
   The reasons to decide the dosage, negative and positive controls, preparing and treatment method of S9mix, procedure of experiment and operation, treatment time, time of making specimens, judgment standard of aberration, judgment standard of test results etc.

(4) Test results
The observed number of cells, appearance ratio of cells with abnormal structure and the number of abnormality per kind, appearance ratio of polyploidy number (including endoreduplication). Polyploidy (including multiplication within the nucleus, data on cell growth (inhibition ratio, mitotic index etc.), whether or not there is deposition, background data etc.

(5) Consideration and conclusion

(6) Reference literatures

Micronucleus test (2-1-19-3)

1. On the species of animals

In case of the micronucleus test using the peripheral blood of the animals except mice, it is necessary to have scientific information showing that the test is equivalent to the test using the bone marrow of the animal. The sensitivity of male mice tends to be higher than that of female when significant difference of micronucleus- inducing ratio is observed between male and female of mice, and accordingly young and mature male mice of 7～10 weeks old shall be used as the animals for the test.

2. On the frequency of administration (On the dosing frequency)

Plural administrations (doses) generally mean two to four administrations per 24 hours. More administrations than that are possible, but use the dosage that causes some kind of toxic symptoms as the maximum dosage.

3. On the dosing steps

(1) The maximum dosage shall be decided by a preparatory test conducted under the same conditions as those of the final test.

(2) In principle, set three dosing steps with geometric ratio under $\sqrt{10}$. In case that no toxicity is observed with the dose of 2,000mg/kg or more and the mutagenicity is not expected according to the characteristics of chemical structure etc., carry out the test with the dosage of 2,000mg/kg and negative control test only.

(3) In case of plural administrations, the maximum dosage shall be 2,000mg/kg for the test of 14 days or shorter, and 1,000mg/kg for the test of over 14 days.

4. On the control

In case of using solvents with no background data, add non-treatment control.

5. On the test method

(1) In case of single administration, the first specimens of bone marrow shall be made 24 hours after the administration or later, and the second ones shall be made within 48 hours after the administration. In case of sampling peripheral blood, the first specimens shall be made 36 hours after the administration or later, and the second ones shall me made within 72 hours after the administration. The specimens for the maximum dosage shall be made as the second specimens.

(2) In case of plural administrations, each one specimen shall be made for bone marrow

and peripheral blood within 18～24 hours and within 24～36 hours after the last administration respectively. The period of making peripheral blood specimen is a little different from that of the OECD guideline, but there are many kinds of substance that show their maximum frequency 30±6 hours after the administration according to the data of Higashikuni etc. that are referred to for the OECD guideline.

6. On the observation

(1) As the staining methods of specimens, acridine orange fluorescence staining or Giemsa staining is used for bone marrow specimens, and acridine orange supravital staining is used for peripheral blood specimens. Juvenile erythrocyte (red blood cell) means so-called polychromatic red blood cell in the case of Giemsa staining, and RNA-containing erythrocytes whose cytoplasma emits red fluorescent light at the acridine orange fluorescence staining and reticulocytes which can be distinguished by supravital staining.

(2) As to the animals dosed for over 4 weeks, 2,000 or more mature erythrocytes per capita shall be observed and the micronucleus appearance frequency shall be recorded.

7. On the judgment of the test result

Statistical method shall be used as an auxiliary step, and the final judgment shall be collectively made taking biological meaning into full consideration.

8. On the report

In principle, show the observed results per individual animal and the data per group (average value or standard deviation).

Following items shall be described in the report.

(1) Information on the test substance
Same as that for the acute oral toxicity test

(2) Information on the animals used for the test
Same as that of the acute oral toxicity test

(3) Information on the test conditions
The reason to decide the dosage, dosing route, administration frequency, dosage, specimens making period and making method, negative and positive control (substance and method), staining method, observation method of micronucleus etc.

(4) Test results
Appearance frequency of the juvenile erythrocytes having micronucleus and the number of observed cells, the ratio of juvenile erythrocytes to all erythrocytes (%) and the number of observed cells, method of statistical transaction and result, background data etc.

(5) Consideration and conclusion

(6) Reference literatures

Pharmacology test

Pharmacology test (2-2-1)

1. Base of the design of test

When the toxicity information concerning the route is already known, the information can be used. The general symptoms shall be observed quantitatively and diachronically by multi-dimensional observation method to grasp the characteristics of the poisoning.

On the whole, full attention shall be paid not to use single species of test animal. And design test methods so that the sexual differences can be considered. And in order to consider treatment method theoretically, assume the method of acute toxic action and investigate the influence of possible antagonist depending on the necessity.

In case of using anesthetic animals, carry out the test taking the absorption change of test substance into consideration since the absorption of the test substance differs depending on the dosing method. Intravenous dosage is generally used when anesthetic animals are used.

2. On the items to be inspected

Following items shall be inspected depending on the acute toxic degree.

(1) When the acute toxicity is stronger than the degree equivalent to that of deadly poison, full examination shall be conducted as to the whole inspection items, and the antidote or lifesaving method shall be prepared.

(2) When the acute toxicity is weaker (oral LD_{50} is larger than 2000mg/kg), examine the minimum inspection items (situation observation, influence on respiration and blood pressure).

(3) When the acute toxicity is different from above (1) and (2), examine the inspection items depending on the degree of acute toxicity and the characteristics of toxicity emergence and check the antidote.

Concerning the items which should be generally tested, it is not necessary to test the items if their influence can be well assumed by using the results of other toxicity tests. And besides, as to the items which should be tested additionally depending on the necessity, it is not necessary to test the items if necessary information for them is already obtained from the results of other toxicity tests.

3. On the report

Following items shall be described in the report, and consider the possibility of acute poisoning, its characteristics and its type on the basis of the obtained test results and the information that is already known.

(1) Information on the test substance
Same as that of the acute oral toxicity test

(2) Information on the animals used for the test
Same as that of the acute oral toxicity test

(3) Information on the test condition
Preparing method of the test substance, the reason to set the dose, dosing route and period, dosage, dose intervals in the case of gradually increased dosing etc.

(4) Test results, the method and result of statistical transaction

(5) Consideration and conclusion

(6) Reference literatures

<Test on metabolism in animals, metabolism in plants, behavior in soil and behavior in water >

Definition

1. [Test substance etc.]: defined as the total of test substance and its metabolite (the total radioactivity in case of radioactive labeled substance)

2. [Identification]: defined as the complete decision of chemical structure that is decided by using spectral analysis or co-chromatography analyses with standard ones or identified metabolites in the several chromatography analyses with different principles.

3. [Chemical characterization]: defined as the designation whether there is polarity, and basic framework of active ingredients, confirmation of the difference or similarity with the metabolites in the animal bodies, plant structure, soil or water even when the complete identification is not realized. This also means tentative identification when it is identically same as a standard one obtained only by a kind of chromatography, the identification of decomposition products by chemical change etc. and clarification of the characteristics by designation of molecular weight only.

Test on metabolism in animals

Test on metabolism in animals (2-3-1)

The test shall be conducted with reference to the guideline. However, appropriate methods can be studied according to the properties of the test substance and test methods can be properly added, reduced or replaced with other methods. And furthermore, when proper data are obtained from the toxicity tests concerning the kinetics of test substance in the bodies, the data can be used.

1. On the animals used for the tests

Pregnant animals shall be used, too, when toxic symptoms of pregnant animals are significantly different from the results of general toxicity test and the toxicity through placentas is hinted as the result of reproductive toxicity test and teratogenicity tests.

2. On the method of administration

(1) The tests of intravenous dosage shall be conducted, too, when it is difficult to properly grasp body distribution and metabolism of the absorbed test substance due to the fact that the absorption ratio in the case of oral dosage is significantly low etc.

(2) The test substance accumulation is considered if the half time of the test substance concentration in the blood plasma at the single dose test is found to be longer than 48 hours and the half time of the test substance etc. in organs or tissues is hinted to be clearly longer than that in the blood plasma. In such cases, it is preferable to conduct repeated dose test, too.

(3) Use labeled chemical compounds in principle for the repeated dose test to check the accumulation of the test substance. Following information shall at least be obtained by this test.
The concentrations of the test substance etc. at plural times after the end of repeated dose period and their ratio to that after the single administration concerning following organs and tissues.
- Blood plasma, organs and tissues on which the accumulation is considered.
- Organs and tissues on which toxic effect such as pathological change is observed (suspected) as the result of 90 days repeated dose test, one year repeated dose test or carcinogenic test.

3. On the establishing of the number of test animals and the test groups

(1) In case of rodent animals, the number of the animals is four or more per sex and dosage for the assessment of excretion and material balance, and it is in principle three animals or more for the time course investigation of body-internal distribution and blood concentration. The number of non-rodent animals can be smaller than that for rodent animals.

(2) The "high dosage" is defined as the dose that causes toxic effect at the single dose and repeated dose but does not cause the death of animals. A standard value of the upper limit is 1,000 mg/kg.

(3) Repeated "high dosage" tests shall be executed when the toxic symptom seemingly important to assess the toxicity is observed but it is difficult to understand the occurrence of toxicity only by the results of single dose test and repeated low-dosage test.

4. On the items to be investigated

(1) Absorption
① Absorbed amount (the ratio to the dosage) can be calculated using following methods for example.
a. Calculate as the total sum of the excretion to urine and expired air (the ratio to the dosage) and the residual amount within the body (the ratio to the dosage) in case that the excretion to faeces is little.
b. Calculate the total amount of the test substance etc. in urine and gall and the residual amount in the body of the animals that are cannulated in the bile duct.
c. Calculate by comparing the discretion amount of the test substance to the urine (the ratio to the dosage) or the area under the curve (AUC) of the test substance measured after the intravenous administration and those measured after the oral administration
② Following information shall be at least obtained at the test of "transition of the chemicals concentration in the blood".
a. The maximum concentration of the test substance etc. (C max).
b. The time until the concentration of the test substance reaches Cmax after the administration.
c. The AUC of the test substance etc.
d. Half-times of test substance etc.

(2) Distribution
① "Major organs and tissues" include sexual gland, adrenal gland, thyroid gland and other endocrine organs, brain, lung, heart, liver, kidney, spleen, uterus, gastrointestinal tract, blood, muscle and fat etc.
② "Organs and tissues which toxic influence were observed on" mean the organs and tissues on which pathological change etc. due to the administration of the test

substance were observed as the result of 90 days or one year repeated dose test, carcinogenic test etc.

③ As to the blood analysis to examine the distribution of the test substance etc. in the blood, separate the blood into blood plasma and hemocytes, and analyze both individually, or analyze both the whole blood and blood plasma separately.

④ "Appropriate plural time points" means in principle more than three time points.

⑤ "Distribution ratio" means the ratio (%) of the test substance etc. distributing in the object organs or tissues to the total dosage. The calculation of the distribution ratio can be neglected for the tissues (fat, blood etc.) when it is difficult to sample all of the tissues.

⑥ Auto-radiography of the whole body is an auxiliary equipment to obtain the information on the distribution of the test substance etc. in the organs and tissues for which it is difficult to clarify the information by sampling and examining.

(3) Excretion

① Gathering of expired air is not needed for the main test when significant amount of the test substance etc. (1% or more of the radioactive that was dosed until 24 hours after the administration) is not observed at the preliminary test.

② The excreted amount into gall shall be measured when 20 % or more test substance is excreted into faeces and the toxicity by the metabolite in the gall is suspected as the result of other tests.

③ The excreted amount into lactation shall be measured when the toxicity due to the excretion of test substance etc. into the lactation is suspected in such cases as that the born offspring are not growing well.

④ After the measuring period of the discretion is over, slaughter the test animals, measure the amount of residual test substance etc. in their bodies and clarify the material balance of the test substance etc. The material balance shall be 90 to 110 %.

Enterohepatic circulation shall be investigated when the gall is major discretion route.

"Material balance" means the total recovery ratio (%) of the test substance dosed to the individual animal body. (the ratio of excreted amount from the bodies and residual amount in the bodies of the test substance etc. to the total dosage)

(4) Metabolism

In principle, identify the metabolite equal to or more than 5 % of the dosed amount in the discretion. It is sometimes necessary to identify the metabolite even when it is less than 5 % if necessary for the assessment of the toxicity.

(5) Others

In order to obtain the information on the metabolism regarding the toxicity, biochemical inspection shall be conducted on the influence to metabolic enzyme systems, the decrease of non-proteineous SH chemical compound etc. depending on the necessity.

5. On the report

In principle, following items shall be described in the report.

(1) Information on the test substance

Same as that of the acute oral toxicity test, and labeled positions and their grounds, degree of radiochemical purity, specific radioactivity etc. {same or equivalent information on the reference substance (reference standard of metabolite etc.) in case of identifying the metabolite}

(2) Information on the animals used for the test

Same as that of the acute oral toxicity test

(3) Information on the test condition

Specifications and preparing method of the dose liquid, toxicity information of the carrier, the amount actually dosed to the animals, calculation method of the amount and detailed information on other method used

(4) The number of the animals and test groups
Dose and the ground to set the dose (including the dose intervals and period in case of conducting repeated dose), the arrangement of test groups, the time of slaughter, the times and intervals for sampling test material

(5) Test results

(6) Consideration and interpretation
This should include assumed absorption ratio and assumed metabolic pathway.

(7) Reference literatures

Test on metabolism in plants

Test on metabolism in plants (2-4-1)

1. On the plants used for the test
Cultivate the plants for the test under the condition that the influence of photolysis can be considered.

2. On the treatment method
(1) Different, plural treatment methods mean for example soil treatment and foliar application (including partial application). However, if other tests can be used to grasp the route of the metabolite etc. more properly, partial application to fruits (seeds) and leaves or single treatment can be used.

(2) When the test is unavoidably conducted without using formulation, the reason for it shall be clearly described.

(3) The equivalent amount range to the used amount of the object agricultural chemicals means 2 to 3 times.

(4) When the amount of metabolite is too little to identify, carry out the test using the amount that enables the identification.

(5) Systematic treatment (体系処理) can be used when plural use methods are planned and it can better clarify the metabolite and metabolizing route to use the plural use method.

3. On the extraction of test material
The crops of long harvest period means such plants as tomato and cucumber that have anthesis and fructification continuously after the beginning of harvest.

4. On the analysis
Clarify in particular the movement to the edible parts for human beings and animals

when analyzing the existence of the metabolites per test material.

5. On the identification of metabolite etc.

(1) Consider the identification ratio of the residual amount of the object metabolite or the total residual amount and other metabolites etc., in chemical characterization.

(2) If the test substance has isomer, confirm whether or not there is the change of the abundance ratio of the isomer.

6. On the report

In principle, following items shall be described in the report.

(1) Information on the test substance

Chemical name, chemical structure, labeled position, purity (radiochemical purity, chemical purity) and the method to maintain the purity

(2) Information on control substance (standard synthesized metabolite etc.)

Chemical name, chemical structure, chemical purity and the method to maintain the purity

(3) Information on the plants used for the test

The cultivar of the plants etc.

(4) Crop cultivation environment

Cultivation status, wavelength distribution and strength of light when artificial light is used, temperature and humidity, the time (season) and weather in case a test is conducted in the field, especially information on the rainfall

(5) The application method used for the test

The ground to decide the concentration (relationship with traditional concentration), the application methods used for the test (used solvent, treatment route to the test system, the number of treatment and treatment intervals) and the information on the radiochemical purity of the test substance when it is used.

(6) Picking of test sample

Stage of growth, the parts of picked samples, the method of separating the sample from the plants, the number of days after the treatment when the samples are picked, intervals of picking

(7) Storage method of samples and storage length

(8) Radioactivity measuring method

(9) Analysis method of metabolites etc.

(10) Example of calculations (including the quantification of metabolites and decomposition products)

(11) The equipment used for the test

(12) The distribution of total residual amount

(13) Extraction ratio

(14) Residual amount after extraction

(15) Amount of major metabolites and their distribution in the edible part

(16) Identification result and chemical characteristics of the major metabolites

(17) Storage stability

(18) Assumed major routes of metabolizing and decomposition

(19) Consideration

Test on behavior in soil (2-5-1～3)

It is possible that agricultural chemicals receive metabolism (biological decomposition) by microorganism in the soil and non-biological decomposition catalyzed by clay minerals. Both decompositions are called "metabolism" in this test.

Definition

1. "Aerobic": is defined as the situation of abundant molecular oxygen

2. "Substance balance": is defined as the total recovery ratio of the test substance treated by the test system (the ratio to the total sum of the amount of the test substance etc. collected as volatile substance and residual amount in the soil and flooded water to the treated amount)

3. "DT_{50}, DT_{90}": is defined as the time length until when 50 % and 90 % of the test substance disappear respectively

4. "Fresh soil": is defined as the soil where microbial activity is maintained.

Test on behavior in flooded aerobic soil (2-5-1)

In the environment of flooded paddy fields, the plow layer is covered with water and the soil layer of several mm to 1 cm from the surface has aerobic environment, and the lower layer has reductive environment though the rooting zone includes oxidative environment. The fate test in flooded aerobic soil shall be conducted in order to clarify the metabolism in the water-flooded paddy field.

1. On the soil used for the test

(1) It is preferable to use the soil that can represent the conditions of the field where the objective agricultural chemicals are used. Provide the soil that has the information on soil particle composition, soil properties classification (FAO/USDA etc.), soil pH (water, KCl water solution, $CaCl_2$ solution), organic carbon content, CEC (cation exchange capacity), major clay minerals and other characteristics useful for the assessment of the test results. Detailed information (including chronical one) of the

place where the soil is collected is needed, too. The information on the soil groups (soil series groups) or their origins will be one of the information useful for the assessment of the test result and accordingly this shall be preferably confirmed.

(2) In case of storing soil, it is preferable to store it in a dark and cold place having 4 ± 2 ℃ atmospheric temperature in order to maintain natural microorganism activity from collecting until using it for the experiment. The storage period must not exceed 1 year.

(3) When it is difficult to sieve the soil by using 2 mm sieves, a sieve of larger mesh can be used, but the mesh size must not be larger than 5 mm.

(4) It is preferable in principle to use an autoclave in case of sterilizing the soil used for the test.

2. On the test condition

(1) In principle, the test temperature shall be 25 ℃, but it is accepted to set a specific temperature (± 2 ℃) from 20 to 30 ℃ at the final test in case that using this condition is considered to be more appropriate in order to clarify metabolic pathway, kind of metabolite and mass balance.

(2) The depth of soil shall be large enough to form reduction layer.

(3) The formation of reduction layer shall be confirmed by the fact that the oxidization-reduction potential at the lowest position of the layer becomes lower than 200 mV.

3. On the execution of the test

(1) In principle, use a solvent that does not affect the microorganism phase when using solvents for the treatment of the test substance. Do not use solvents having fumigation action. (chloroform, dichloromethane and other organic halogen compound)

(2) Calculate the treatment concentration by assuming the tentative specific gravity of soil as 1.0.

(3) It is desirable to carry out the test using more amount than the maximum use because it is useful to identify the metabolites.

4. On the items to be investigated

(1) Distribution

The target mass balance shall be 100 ± 10 % of the treated amount.

(2) Metabolism

In principle, identify, as far as possible, the metabolites that are more in amount than 10 % of the treated amount during the test. Other metabolites near this level shall be chemically characterized. It is desirable to identify the metabolites less than 10 % of treated amount since their identification sometimes becomes necessary for the assessment of the safety.

When the bound residue is formed as the ratio over 20 % of the treatment amount, carry out the complete extraction using characterized analysis of the residue (for example, heat extraction like Soxhlet extraction, etc.) and that using acid and alkali solvent, etc.

When the test substance is mixture of stereoisomers and significantly remains in the

test soil, it is preferable to obtain the information on the change of the ratio of stereoisomers.

5. On the report

In principle, following items shall be described in the report.

(1) Information on the test substance
Chemical name, chemical structure, labeled position, purity (radiochemical purity and chemical purity) and storage condition

(2) Information on the reference substance (standard synthesized metabolite etc.)
Chemical name, chemical structure, chemical purity and storage condition

(3) Test conditions
The place of collecting soil, the time of collection, soil characteristics and measuring method by which the characteristics was decided, storage condition, storing period, sieving method,
test container
Incubation conditions (including pre-incubation)
Formation of the reduction layer (measuring method of oxidization-reduction potential and results)
Collecting method of volatile substance
Depth of the flooded water and its maintaining method
Nominal temperature and actually measured temperature
The thickness and the weight of the soil in the test containers

(4) Treatment
Nominal concentrations and the reason (the relation with the maximum conventional dose amount)
Treatment method (including the liquid used for the treatment of soil)
Radiochemical purity of the test substance when treated

(5) Collection of test samples and their analysis
The time of collecting the samples
Analytical method
Measuring method of the radioactivity
Calculation method of the information on the disappearance (DT50 and DT90)
Examples of calculation (including the quantitative calculation of the metabolites)

(6) Results
Distribution and mass balance
Extraction ratio
Formation ratio of the major metabolites
The results of identification and characterization of major metabolites
Formation ratio of volatile substance
Formation ratio of bound residue
Information on the disappearance of the test substance (DT50 and DT90)

(7) Consideration and interpretation (including assumed metabolic pathway)

(8) The storage method, period of storage and storage stability of test samples when needed.

Test on behavior in aerobic soil (2-5-2)

The plowed soil, excluding that of paddy field, generally has aerobic circumstance, and oxidative metabolism by soil microorganisms dominates there. The fate in aerobic soil test shall be conducted in order to clarify this kind of metabolism.

1. On the soil used for the test

Same as that for the Test on behavior in flooded aerobic soil (2-5-1)

2. On the test conditions

(1) In principle, the moisture content of soil shall be measured and water shall be added to the soil when needed.

(2) When the test substance is volatile like soil fumigant, etc., the use of non-aeration test system may be more appropriate.

(3) Other conditions are same as those for the Test on behavior in flooded aerobic soil (2-5-1).

3. On the execution of the tests

Same as those of the Test on behavior in flooded aerobic soil (2-5-1)

4. On the items to be investigated

Same as those of the Test on behavior in flooded aerobic soil (2-5-1)

5. On the report

In principle, following items shall be described in the report.

(1) The moisture content in the soil (the percentage to the maximum water holding capacity) and the moisture maintaining method

(2) Other items are same as those of the Test on behavior in flooded aerobic soil (2-5-1). However, the formation of the reduction layer (the method and result of measuring oxidation-reduction potential) and flooding water depth and its maintaining method shall be excluded.

Test on behavior in anaerobic soil (2-5-3)

The soil layer existing under plowed soil has generally an anaerobic circumstance, and there may be kinds of metabolism that are different from aerobic metabolism. As to agricultural chemicals with high soil permeability, the metabolism in this soil layer must be clarified, and accordingly the Fate in Anaerobic Soil Test shall be executed.

1. On the soil used for the test

Same as that of the Test on behavior in flooded aerobic soil (2-5-1)

The formation of reduced layer shall be confirmed by checking the oxidation-reduction potential at the layer deeper than 2 cm from the soil surface to be 0 mV (target value).

2. On the test conditions

Same as those of the Test on behavior in flooded aerobic soil (2-5-1)

3. On the execution of the test

Same as that of the Test on behavior in flooded aerobic soil (2-5-1)

4. On the items to be investigated.

Same as those of the Test on behavior in flooded aerobic soil (2-5-1)

5. On the report

Following items shall be described in the report.

(1) Incubation conditions (including pre-incubation and the kinds of inert gases)

(2) Other items are same as those of the Test on behavior in flooded aerobic soil (2-5-1).

Test on behavior in water (2-6-1, 2)

Definitions shall comply with test on behavior in soil.

Test on hydrolytic behavior (2-6-1)

1. On the Test Water

Use the buffer solutions of pH where decomposition was recognized.

As decomposition substances would vary on pH, conduct the test at any pH where decomposition was recognized.

Measure pH of the buffer solution at test temperature with at least ±0.1pH accuracy.

The term "decomposition is recognized" herein refers to the case where the half-life at 25 ℃ is less than one year.

2. On the Test conditions

The test container shall be sealable. In principle, metal containers are not acceptable.

Air trapping is required when the sufficient mass balance is not obtained due to volatile transformation products. If the sufficient mass balance is obtained only by collecting water, air trapping is not necessarily required.

When air trapping is not required, preferably reduce the space above test solution in the test container as much as possible. When conducting air trapping, take care such that the air layer does not supply oxygen.

"±1 ℃" of test temperature refers to the control range from the test nominal temperature.

When the test substance is less soluble in water, a cosolvent is also applicable to prevent adherence on the inner surface of the container. In this case, the solvent concentration shall be no more than 1% in principle.

Exclude oxygen by, for example, blowing nitrogen or argon and frothing for five minutes prior to preparation of the solution.

3. On the Points to investigate

(1) Mass balance

A desirable mass balance is 100±10% of the treated amount.

(2) Transformation products

In principle, identify or chemically characterize transformation products formed no less than 10% of the treated amount throughout the experimental term. Even if transformation products are less than 10%, identification would be required when necessary for safety evaluation. Transformation products formed no less than 10% of the treated amount shall be defined as the main transformation products. When the test substance is optical isomer and substantially remains in the sample, preferably obtain information whether the abundance ratio of the optical isomer varies.

4. On the Report

In principle, the report shall include the items listed below.

(1) Information of the test substance

Chemical name, chemical structure, label position, purity (radiochemical purity, chemical purity) and storage condition

(2) Information of the reference substance (synthetic transformation product standard, etc.)

Chemical name, chemical structure, chemical purity, and storage condition

(3) Test conditions and the test substance treatment

Test container

Composition of the buffer solution, concentration, pH and the amount of the test solution in the test container

Nominal concentration and the reason (relationship with solubility in water) and the measured concentration immediately after the treatment

Nominal temperature and the measured temperature

Exclusion of any decomposition factors from the test substance other than hydrolysis (shading, sterilization, deoxydation, etc.)

Treatment method (cosolvent concentration in the test system when applying a cosolvent for the treatment)

Radiochemical purity of the test substance at the treatment

(4) Sampling and assay

Collecting time of samples

Analysis method

Measurement method of radiation

Calculation method of elimination information (DT50 and DT90 if possible)

Calculation examples (including quantitative calculation of transformation products)

(5) Results

Mass balance

Formation rate of each main transformation product

Identification and chemical characterization results of main transformation products

Formation rate of volatile substances when formed

Elimination information of the test substance (DT50 and DT90 if possible)

Fate information of the main transformation products (DT50, DT90) if possible

(6) Consideration and interpretation (including expected hydrolytic pathways)

(7) Stock method, stock period and stock stability of samples as appropriate

Test on photolytic behavior in water (2-6-2)

1. On the Test Water

(1) Use distilled water (or buffer solution) of which the items are specified such as: preparation method; pH; conductivity; dissolved oxygen amount and absorption spectra at the 290-750nm wavelength range as appropriate; and other properties for evaluation of study results.

(2) Use natural water of which the items are specified such as: pH; dissolved oxygen amount; suspension substance amount; total amount of volatilized residue; conductivity; absorption spectra at the 290-750nm wavelength range; other properties useful for evaluation of study results; and detailed information of the sampling point and time.

(3) Water prepared by flooding soil or sediment is included in natural water.

(4) Take care not to deteriorate natural water when stored.

(5) Sterilization methods of natural water include autoclaving or sterile filter treatment.

(6) If the test substance is unstable depending on pH, the buffer solution (at stable pH) is applicable in place of distilled water.

(7) Humic acid solution, etc. are applicable in place of natural water if they are more suitable to specify the photolysis pathways, the transformation product type and the mass balance of the test substance in the test.

2. On the Test conditions

(1) In principle, use a xenon lamp with 290nm or shorter wavelength cut as a test light source of artificial lighting that is similar to sunlight that would reach the ground in terms of wavelength distribution. Natural sunlight is also applicable if the accurate light intensity and the measurable wavelength range are available.

(2) Avoid containers whose light incidencing side is made of ordinary glass.

(3) Air trapping is required when the sufficient mass balance is not obtained due to volatile transformation products. When the sufficient mass balance can be obtained only by collecting water, air trapping is not necessarily required.

(4) "±2 ℃" of test temperature refers to the control range from the test nominal temperature.

(5) A cosolvent is applicable when the test substance is less soluble in water to prevent absorption on the inner surface of the container. In this case, the solvent concentration shall be no more than 1% in principle. However, avoid a cosolvent known to have a photosensitization (acetone, etc.) or extinction property.

3. On the items to investigate

Same as those of test on hydrolytic behavior (2-6-1)

4. On the Report

In principle, the report shall include the items listed below.

(1) Test substance information

Chemical name, chemical structure, label position, purity (radiochemical purity, chemical purity) and storage condition

(2) Reference substance information (synthetic transformation product standard, etc.)

Chemical name, chemical structure, chemical purity and storage condition

(3) Test conditions and treatment of test substance

Preparation method and properties of distilled water (or buffer solution)

Sampling point, sampling time, and property-compatible measurement method, stock method and stock period of natural water

Preparation method of buffer solution and the reason of use when using buffer solution in place of distilled water

Preparation method and the origin of soil/sediment when using natural water prepared by flooding soil or sediment

Preparation method of solution when using humic acid solution, etc. in place of natural water;

Sterilization method and sustainment of sterilization

Irradiation source (Wavelength distribution information of incidencing light according to measurement information or information provided by irradiation equipment manufacturer);

Irradiation method

Light intensity (W/m^2) and the measured wavelength range (nm)

Test container

Light path length of water in the test container

Nominal temperature and the measured temperature

Nominal concentration and the reason (relationship with solubility in water) and the measured concentration

Treatment method (cosolvent concentration in the test system when using a cosolvent for the treatment)

Radiochemical purity of the test substance at the treatment

(4) Collection and assay of samples

Sampling time

Analysis method

Radiation measurement method

Calculation method of elimination information (DT50 and DT90)

Calculation examples (including quantitative calculation of transformation products)

(5) Result

Mass balance

Formation rate of each main transformation products

Identification and chemical characterization results of main transformation products

Formation rate of volatile substances when formed

Elimination information of the test substance (DT50 and DT90 if possible)

Expected elimination information (DT50) under natural sunlight (at latitude 35 degrees N (Tokyo) in spring (April-June))

Formation and decline information of main transformation products (DT50 and DT90)

if possible

(6) Consideration and interpretation (including expected photolysis pathways, and effects on photolysis when using humic acid solution, etc. in place of natural water)

(7) Storage condition, stock period and stock stability of samples as appropriate

[Reference]
Expected examples of the half-life in water under sunlight are shown below.

1. Flux of global solar radiation

The daily integrated value of the flux of global solar radiation of Tokyo (from Scientific Chronology 1998: Average of integrated value of 1974-1990) is listed below.

April	May	June	Average
14.3	16.0	13.6	14.6 ($MJ/m^2/d$)

- Flux of global solar radiation refers to the total amount of the flux of direct solar radiation and the flux of scattering solar radiation.
- The measurement device is an electric pyrheliometer (with measurable wavelength range about 300-2800nm)

2. Spectroirradiation illuminance distribution of sunlight

Sunlight reaching the ground varies on such factors as latitude, season, time, air pollution degree, water vapor amount,etc, and the variation depends on the wavelength as well. As the spectral property of sunlight radiation is constantly changing, the spectral distribution of sunlight is hard to standardize.

Accordingly, use the spectroirradiation illuminance distribution of reference sunlight defined in "Secondary Reference Crystalline Solar Cells (C8911-1998)" of the Japanese Industrial Standard (JIS).

3. Expected half-life in water under sunlight

- When the daily integrated value of flux of global solar radiation is employed as Io, the average of April-June is:

$$Io = 14.6\ (MJ/m^2/d) \qquad (1)$$

- When the light intensity of the artificial light source used in the test is employed as IL-H (measurable wavelength range: L-H nm, W/m^2), the irradiation illuminance of sunlight (Is) at the L-H nm wavelength range is represented by Formula (2) according to the spectroirradiation illuminance distribution of reference sunlight (JIS C8911-1998).

$$Is = Io \times (\text{L-H nm irradiation illuminance}) / (\text{Irradiation illuminance at all wavelengths}) \quad (2)$$

- When the half-life of chemical substances at the light intensity IL-H is employed as DT50lab(d), the integrated value of irradiation illuminance IDT50 (MJ/m^2) from the commencement of the test to half-life is represented by the following Formula.

$$IDT50 = IL\text{-}H \times DT50lab \times 24 \times 3600 \times 10^{-6}\ (MJ/m^2) \quad (3)$$

・Accordingly, the half-life under sunlight DT50sun(d) is represented by Formula (4) .

$$DT50sun = IDT50 / Is \quad (4)$$

<Examples>
When conducting the test under the following conditions, calculate as shown below.

- The light intensity of the artificial light source (I300-400): 30W/m^2 (constant throughout the experimental term)
- The measurable wavelength range of the light intensity: 300-400nm

Assume that the half-life of chemical substances under these conditions, DT50lab, is 10 days.

According to JIS C8911, the ratio of（300-400nm irradiation illuminance）against irradiation illuminance at all wavelengths is 4.6%. Thus, 300-400nm irradiation illuminance of sunlight (monthly average) of Tokyo in spring (April-June) calculated based on Formula (2) is:

$$Is = Io \times (\text{300-400nm irradiation illuminance})/(\text{irradiation illuminance at all wavelengths})$$
$$= 14.6 \times 4.6\% = 0.672 \ (MJ/m^2/d)$$

The integrated value of irradiation illuminance from the commencement of the test to the half-life calculated based on Formula (3) is:

$$IDT50 = 30 \times 10 \times 24 \times 3600 \times 10^{-6}$$
$$= 25.92(MJ/m^2)$$

Accordingly, the half-life of under sunlight DT50sun based on Formula (4) is:

$$DT50sun = 25.92 / 0.672$$
$$= 38.6 \text{ days}$$

Test on toxicity on aquatic animals and plants (2-7-1~7)

Fish acute toxicity test (2-7-1-1)

1. Test organisms

(1) Organism species

① A TGAI must be studied using *cyprinus carpio* or *oryzias latipes*. Preferably, also study the formulation using *cyprinus carpio* or *oryzias latipes*. Specify the source, breeding method, etc. of the test organism.

If additional organism species of fishes are required to be studied, study a TGAI using any one of species consisting of l*epomis macrochirus*, *oncorhynchus mykiss*, *poecilia reticulata*, *danio rerio* and *pimephales promelas*.

② Preferably, use the standard substance to confirm the reproducibility of the test. Conduct the test using the standard substance for each test using the test substance, for each group of the same organism (the same source) or for each certain period (at least twice a year). PCP-Na (pentachlorophenol sodium) and copper (II) sulfate are acceptable as the standard substances. However, note that pH of PCP-Na and water hardness of copper (II) sulfate would vary the toxicity. Waste leftover solutions for preparation of chemicals and used chemicals after proper treatment.

Record LC50 with the standard substance along with the background data (average ± standard deviation) in the study report.

(2) Acclimatization

The standard acclimatization period is given in the figure below.

	12 days before	9 days before	7 days before	1 day before	test day
Obtaining					Not feeding
		Stable stage	Mortality rate research		
		Acclimatization under the test environmental conditions			

2. Setting of test concentration areas

(1) In principle, the separation factor shall be within the range of 1.3 - 2.2.

(2) The maximum test concentration for a TGAI shall be 100mg/L in principle. The formulation shall be studied as far as possible with the maximum concentration 1,000mg/L as the principle formulation concentration. If test substance related effect is not observed at the maximum concentration, the test concentration area may be one concentration.

3. Preparation of test solution

(1) When using a TGAI that is not water soluble, prepare the test solution by mechanical dispersion (e.g., ultrasonic treatment) or using a commonly-used solubilizing agent (e.g., N,N-dimethylformaldehyde, triethyleneglycol, acetone, ethanol, methanol, hardened castor oil). In this case, the agent should be uniformly dispersed, but is not necessarily dissolved thoroughly.

(2) Two or more kinds of solubilizing agents may be combined. In this case, the solubilizing agent concentration in the test solution shall be constant for all the test concentration areas in principle and preferably no more than 100mg/L (or 0.1ml/L).

4. Environmental conditions

(1) Light

Intensity or quality of the light is not particularly specified herein. In principle, the light condition commonly used in the laboratory is acceptable.

(2) Dilution water

① Preferably, use water with the total hardness 10-250mg $CaCO_3$ /L and pH6.0 - 8.5.

② When using reconstituted water, prepare it using a special or analytical grade reagent and using distilled water or deionized water with conductivity no more than $10\mu\ Scm^{-1}$.

③ When using tap water or natural water to prepare dilution water, describe the source and the pretreatment method. When using reconstituted water, describe the composition.

④ Examples of reconstituted water are given below.

Examples of reconstituted water (ISO6341-1982)

(a) Calcium chloride solution

Dissolve 11.76g $CaCl_2$-$2H_2O$ in deionized water to prepare 1L solution.

(b) Magnesium sulfate solution

Dissolve 4.93g $MgSO_4$-$7H_2O$ in deionized water to prepare 1L solution.

(c) Sodium hydrogen carbonate solution

Dissolve 2.59g $NaHCO_3$ in deionized water to prepare 1L solution.

(d) Pottasium chloride solution

Dissolve 0.23g KCl in deionized water to prepare 1L solution.

Blend 25ml of each (a)-(d) solution to prepare 1L deionized water in total. This solution includes 2.5mmol/L calcium and magnesium ion. The ratio of Ca against Mg ion is 4:1 and the ratio of Na against K ion is 10:1.

(3) pH

In principle, the pH range suitable for breeding fish is about 6.0 to 8.5. If pH exceeds this range due to addition of the test substance, pH of the test solution may not be adjusted.

5. Observation and measurement

(1) Observation of general conditions of test fish

Record whenever observing such conditions as: loss of equilibrium; abnormal behaviors; upturned nose; bleeding; curved spine; ruffled scales; and discoloration.

(2) Measurement of test substance concentration

① When using a TGAI as the test substance, measure the concentration of each test concentration areas in order to confirm the test substance concentration in the test solution and obtain information of the concentration stability.

② In the flow-through studies or the static studies, conduct measurement at least at the commencement, 48 hours after the commencement and at the end of exposure. However, measurement 48 hours after the commencement may be omitted when the test solution concentration is obviously less variable. In the semi-static studies, conduct further measurement before and after changing water.

③ Collect a sample from the intermediate layer of the test solution. Don't stir in principle even if the test substance partially precipitates or floats on the surface layer.

④ When conducting studies using the formulation, the test substance concentration is not necessarily measured.

(3) Measurement of environmental conditions

① When using dechlorinated tap water or natural water as dilution water, preferably conduct water quality inspection prior to the study with reference to the water quality parameters, etc. Water quality inspection may be periodically conducted.

② Measure water temperature, dissolved oxygen concentration and pH of the test solution for all experimental areas. Conduct measurement at least at the commencement and the end of exposure. In addition, conduct further measurement before and after changing water in the semi-static studies. In this case, preferably conduct measurement every 24 hours to confirm the fluctuation range.

6. Result assay method

(1) Common methods for calculating LC_{50} include Probit method, Moving average

method, Binomial method, and Doudoroff et al. method.

(2) When using a TGAI as the test substance and the test substance concentration varies less than ±20% of the nominal concentration or the measured concentration at the commencement of exposure throughout the exposure period, the measurement results may be analyzed using the nominal concentration or the measured concentration at the commencement of exposure respectively.
An average of measured concentration could be calculated as shown below.

① In the static studies

$$\overline{MC} = \frac{Conc\ A - Conc\ B}{\ln(Conc\ A) - \ln(Conc\ B)}$$

$\overline{MC}$: average of the measured concentration
Conc A : the measured concentration at the commencement of exposure (or at the preparation).
Conc B : the measured concentration at the end of exposure
ln(Conc A): natural logarithm of the measured concentration at the commencement of exposure (or at the preparation)
ln(Conc B): natural logarithm of the measured concentration at the end of exposure

② In the semi-static studies
The average of the measured concentration is calculated using the following Formula with the exposure term (from the commencement of exposure to changing water, from changing water to changing water, and from changing water to the end of exposure) assigned.

$$\overline{mc_n} = \frac{Conc\ A_n - Conc\ B_n}{\ln(Conc\ A_n) - \ln(Conc\ B_n)}$$

$\overline{mc_n}$: the average of measured concentration during the exposure term
Conc A_n: the measured concentration at the commencement of exposure or after changing water
Conc B_n: the measured concentration at the end of exposure or before changing water

Commencement of exposure			Changing water			Changing water			Changing water			End of exposure		
A_1		B_1	A_2		B_2	A_3		B_{n-1}	A_n		B_n			
	$\overline{mc_1}$			$\overline{mc_2}$						$\overline{mc_n}$				

Calculate an arithmetic average using the average of the measured concentration during each exposure term as calculated above.

$$\overline{MC} = \frac{\overline{mc_1} + \overline{mc_2} + \overline{mc_3} + \cdots + \overline{mc_n}}{n}$$

③ In the flow-through studies
Calculate an arithmetic average of each measured concentration. (When measured only at the commencement of exposure and at the end of exposure,

n=2.)

$$\overline{MC} = \frac{\text{Conc 1} + \text{Conc 2} + \cdots + \text{Conc n}}{n}$$

Conc n: the measured concentration at each time point

7. Items to be reported

(1) The test method should include the following items.

① Exposure conditions
Exposure method (static, semi-static or flow-through); test nominal concentration and separation factor (the summary of the preliminary test is also essential.); preparation method of the test solution (When using a solubilizing agent, describe the type and the concentration adopted.); exposure term, etc.

② Environmental conditions
Dilution water; test container and device; amount of the test solution; water temperature; light, etc.

③ Observation and measurement items, etc.
Observation items and method; measurement method of the test substance concentration (when using a TGAI as the test substance); water quality inspection items and method; assay method of results, etc.

(2) Study results

① When the test fish showed abnormal symptoms, preferably submit the photos as attached documents.

② Test solution conditions concerning other items include existence or nonexistence of precipitation and/or sedimentation.

(3) When altering the test method, report the altered points and the reason.

Fish (larvae) acute toxicity test (2-7-1-2)

1. Test organisms

(1) Organism species
Specify the source and the breeding method of parent ricefish (*oryzias latipe)*.
Confirm LC_{50} of the standard substance in accordance with Fish acute toxicity studies.

(2) Acclimatization

① In addition to acclimatization that is exactly the larvae producing condition, record environmental conditions including breeding temperature of parent ricefish and light. Considering that one ricefish can supply 20-30 eggs, obtain sufficient parent ricefish in number according to the required number of larvae for the test.

② Preferably, collect fertilized eggs early in the morning to align the hatch day.

③ Incubate fertilized eggs with a strong air blow until hatch such that they can float in water.
However, restrict air blow the day before hatch. (The average of hatch days at 25 degrees C is about 10 days.)

④ Use a glass tube to collect larvae. Considering ricefish's behavior to escape, develop the situation to facilitate collecting by, for example, increasing the ratio of larvae.

2. Setting of test concentration areas

In principle, the separation factor shall be within the range of 1.3 - 2.2.

3. Preparation of test solution

Comply with Fish acute toxicity studies.

4. Environmental conditions

Comply with Fish acute toxicity studies.

5. Observation and measurement

(1) Observation of general conditions of test fish
Record whenever observing the conditions different from the control (e.g., abnormal behaviors, decreased activities).

(2) Measurement of the test substance concentration and environmental conditions
Comply with Fish acute toxicity studies.

6. Result assay method

Comply with Fish acute toxicity studies.

7. Items to be reported

Comply with Fish acute toxicity studies.

Daphnia spp **acute immobilization test (2-7-2-1)**

1. Test organisms

(1) Organism species
① Conduct the test of a TGAI using *Daphnia magna*. Preferably, conduct the test of a formulation using *Daphnia magna*.
When using other species than *Daphnia magna* in the test, confirm that the test result would be equivalent to the one using *Daphnia magna* by conducting the comparison test or by referring to known findings, the preliminary test results and the test of the standard substance. When using other species than *Daphnia magna*, describe the validity in the report.
② Preferably, conduct the test using the standard substance to confirm reproducibility of the test. Conduct the test using the standard substance for each test substance or for each period (at least twice a year). PCP-Na (pentachlorophenol sodium) and potassium dichromate (chromium(Ⅵ); Cr^{6+}) are applicable as the standard substances. However, note that pH of PCP-Na solution would have effects on the test. Waste leftover solutions for preparation of chemicals and used chemicals after proper treatment. The treatment methods of potassium dichromate include the reduced-chemical sedimentation method and ion exchange.
Describe EC_{50} with the standard substance along with background data (average ±

standard deviation) in the study report.

(2) Breeding of parent daphnia
Feed parent daphnia with unicellular green algal species, etc.

2. Exposure period

The exposure period should be 48 hours.

3. Setting of test concentration areas

Comply with Fish acute toxicity test.

4. Preparation of test solution

Comply with Fish acute toxicity test.

5. Environmental conditions

(1) Dilution water
In principle, comply with Fish acute toxicity test. Proposed reconstituted water is ISO6341, Elendt M4 or M7 in the OECD test guideline 202 *Daphnia SP., Acute Immobilization Test* (2004).

(2) pH
Even if pH fluctuates when adding the test substance, don't adjust pH of the test solution.

(3) Other conditions shall comply with Fish acute toxicity studies.

6. Observation and measurement

(1) Measurement of test substance concentration
Comply with Fish acute toxicity studies.

(2) Measurement of environmental conditions
Comply with Fish acute toxicity studies.

7. Result assay method

(1) Common methods for calculating EC_{50} include Probit method, Moving average method, Binomial method and Doudoroff et al. method.

(2) When using a TGAI as the test substance and the test substance concentration fluctuates less than ±20% of the nominal concentration or the measured concentration at the commencement of exposure throughout the exposure period, the nominal concentration and the measured concentration at the commencement of exposure may be used for the result assay respectively.
The calculation method of the average measured concentration shall comply with Fish acute toxicity studies.

8. Items to be reported

Comply with Fish acute toxicity studies.

Daphnia spp (adult daphnids) acute immobilization test (2-7-2-2)

1. Test organisms

Breed 7-day-old daphnia by feeding.
Other items shall comply with Dalphnia species acute immobilization test.

2. Exposure period

Comply with Dalphnia species acute immobilization test.

3. Setting of test concentration areas

Comply with Dalphnia species acute immobilization test.

4. Preparation of test solution

Comply with Dalphnia species acute immobilization test.

5. Environmental conditions

Comply with Dalphnia species acute immobilization test.

6. Observation and measurement

Comply with Dalphnia species acute immobilization test.

7. Result assay method

Comply with Dalphnia species acute immobilization test.

8. Items to be reported

Comply with Dalphnia species acute immobilization test.

Daphnia spp reproduction test (2-7-2-3)

1. Test organisms

(1) Organism species

Use the species in which the 21-day average cumulative number of living offspring generated by one parent daphnia is at least 60.

(2) Life stage

Don't use first delivery offspring.

(3) Breeding of parent daphnia

Feed parent daphnia with unicellular green algal species such as chlorella vulgaris. Feed with about 0.1-0.2mgC/daphnia/day in organic carbon equivalent amount.

2. Setting of number of test organism and concentration area

(1) Number of test organism

① Use as many organisms as possible but at least 10 organisms for each concentration area.

② Divide organisms in appropriate number so that observation can be conducted. If using 10 organisms for each concentration area, preferably place each organism in a separate vessel. The OECD test guideline 211 Daphnia magna Reproduction Test (1998) proposes: in the semi-static test, using 10 organisms (to test 10 organisms in separate containers); in the flow-through test, using 40 organisms (10 organisms x 4 groups); or in case of using no more than 40 organisms in the flow-through test, for example, using 20 organisms (10 organisms x 2 groups or 5 organisms x 4 groups).

(2) Setting of test concentration areas

① In principle, the separation factor shall be within the range of 1.3 - 3.2.

② When referring to results of Acute immobilization studies, preferably employ EC_{50} of Acute immobilization studies as the targeted maximum concentration and a hundredth of EC_{50} as the targeted minimum concentration.

3. Preparation of test solution

(1) Commonly used solubilizing agents currently include N,N-dimethlyformamide, triethyleneglycol, acetone, ethanol, methanol and hardened castor oil.

(2) Combination of more than one solubilizing agents is acceptable. In this case, the concentration of the employed solubilizing agent in the test solution shall be constant for all test concentration areas in principle, preferably no more than 100mg/L (or 0.1ml/L).

4. Environmental conditions

(1) Test solution amount

Preferably, prepare 50-100ml solution per one daphnia. The test solution amount may be increased according to the test substance analysis conditions. In this case, take care of the feeding amount.

(2) Light

The targeted reference illuminance shall be no more than about 1200 lux. While light intensity shall be no more than 15-20 $\mu E.m^{-2}.s^{-1}$ as specified in the OECD test guideline 211 Daphnia magna Reproduction Test (1998), the targeted reference illuminance is given from the standpoint of facilitating measurement. Light quality is not particularly specified herein.

(3) Feeding

Feed with unicellular green algal species such as cultured chlorella vulgaris. The feeding amount shall be about 0.1-0.2mgC/daphnia/day based on the organic carbon content required by parent daphnia. Preferably, conduct feeding every day.

(4) Dilution water

① When using dechlorinated tap water or natural water as dilution water, preferably conduct the water quality inspection with reference to the water quality parameters prior to the test. The water quality inspection may be periodically conducted.

② Preferably, use water with the total hardness 10-250mg $CaCO_3$/L and pH 6.0-9.0.

③ When using reconstituted water, prepare it using a special or analytical grade reagent and using distilled water or deionized water with conductivity no more than

$10\mu Scm^{-1}$.

④ When using tap water or natural water as dilution water, describe the source and the pretreatment method. When using reconstituted water, describe the composition.

⑤ Elendt M4 or M7 is proposed as reconstituted water in the OECD test guideline 211 Daphnia magna Reproduction Test (1998). The preparation method is given in the appendix.

5. Observation and measurement

(1) Observation of general conditions of test organisms

Count the number of alive and dead parent daphnia and remove the dead. Preferably, repeat this process every day, but definitely at least every 48 hours. Preferably, observe and record the size and conditions of parent daphnia, existence or nonexistence of eggs in the brood chamber, aborted eggs and resting eggs periodically.

Count the number of the alive among offspring and record existence or nonexistence of dead offspring (dead young daphnia) and the conditions based on the visual inspection. Conduct this process every day preferably, but definitely at least three times a week. Remove offspring after counting and observation as they are no longer necessarily bred.

(2) Measurement of test substance concentration

① Preferably, prepare samples for measurement by collecting the same amount of each test solution from respective test concentration areas and blending together. However, if uniformity of each test solution can be confirmed according to results of the preliminary test, for example, samples for measurement may be prepared by collecting from one vessel for each test concentration area.

② Collect samples for measurement from the intermediate layer of the test solution. In principle, don't stir even if the test substance partially precipitates or floats above the surface layer.

③ In the semi-static studies, conduct measurement for all test concentration areas. However, when the test substance concentration at the commencement of exposure is demonstrated to be constantly stable (i.e., kept within 80-120% of the concentration at the commencement of exposure), the second and subsequent measurement of the test substance concentration may be reduced to only the maximum concentration area and the minimum concentration area. Conduct measurement before and after changing water at least once a week.

④ In the flow-through studies, comply with the sampling method in the semi-static studies. However, it would be helpful to conduct an additional measurement in the first week (e.g., three times a week) to confirm stability of the test substance.

(3) Measurement of environmental conditions

① Preferably, measure water temperature, dissolved oxygen concentration, hardness and pH of the test solution for each concentration area. At least water temperature should be measured for the control, and the other items should be measured for the control and the maximum concentration area.

② Conduct measurement at least once a week.

6. Result assay method

(1) EC_{50} and 95% confidence limit

Use a statistical method that calculates the 21-day EC_{50} and the 95% confidence limit by assigning the average cumulative number of alive offspring generated (surviving young daphnia) per one alive parent daphnia in the control (or the solubilizing agent control) and each concentration area on a logistic curve and

conducting regression analysis. Confirm the result evaluation by graphical indication or the significance test. The following formula is known to be useful for analysis in many cases. However, the Hormesis model is more useful in some cases.

$$Y = \frac{c}{1 + \left(\frac{x}{x_0} \right)^b}$$

Y = total number of alive offspring generated per one alive parent daphnia in each test concentration area
x = concentration
c = total number of alive offspring generated in the control
x_0= EC_{50}
b = coefficient

(2) LOEC and NOEC

Analyze the comparison of each concentration area and the control using the analysis of one-way variance analysis (ANOVA). Multiple assays (Dunnett's or Williams) are useful. In this case, confirm the significance of ANOVA by graph indication or Bartlett. When this calculation is not significant, conduct ANOVA by replacing the data with the identical variance or conduct weighed ANOVA.

(3) If the test substance concentration throughout the exposure period fluctuates less than ±20% of the nominal concentration or the measured concentration at the commencement of exposure, the nominal concentration or the measured concentration at the commencement of exposure may be used for result assay.

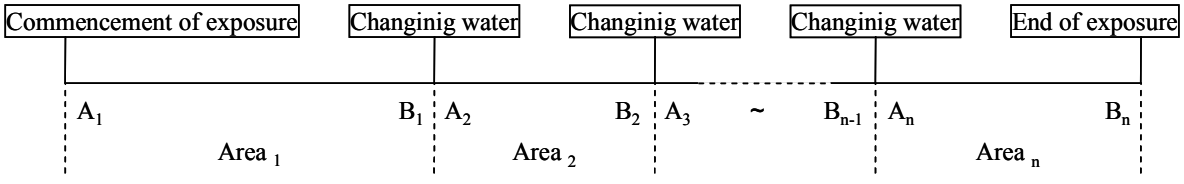
The average measured concentration can be calculated as given below.

① In semi-static studies

Calculate the area below the exponential curve during each exposure period (from the commencement of exposure to changing water, from changing water to changing water, and from changing water to the end of exposure).

$$\text{Area} = \frac{\text{Conc } A_n - \text{Conc } B_n}{\ln(\text{Conc } A_n) - \ln(\text{Conc } B_n)} \times \text{Days}$$

Area : the area below the exponential curve during each exposure period
Days : days of each exposure period
Conc A_n: the measured concentration at the commencement of exposure or after changing water
Conc B_n : the measured concentration at the end of exposure or before changing water
ln : natural logarithm



The average measured concentration shall be obtained by dividing the total area below the exponential curve during each exposure period as calculated above by the number of total days of exposure period.

$$\overline{MC} = \frac{Area_1 + Area_2 + \cdots + Area_n}{\text{Total Days}}$$

② In the flow-through studies
Calculate based on the arithmetic average of each measured concentration.

$$\overline{MC} = \frac{Conc\ 1 + Conc\ 2 + \cdots + Conc\ n}{n}$$

Conc n: the measured concentration at each time point

7. Items to be reported

(1) Describe the following items concerning the test method.
① Exposure conditions
Exposure method (semi-static, flow-through); the test nominal concentration and the separation factor (and the summary of the preliminary test); the preparation method of the test solution (the type and concentration when using a solubilizing agent); the exposure period, etc.
② Environmental conditions
Dilution water; test vessel and devices; test solution amount; water temperature; light; the breeding method (the type and amount of feeding, feeding schedule), etc.
③ Observation and measurement
Observation items and methods; the measurement method of the test substance concentration; measurement items and methods of water quality; the result assay method, etc.

(2) Test results
The term “conditions of the test solution concerning other items” refers to existence or nonexistence of precipitation and/or sedimentation.

(3) When altering the test method, report the altered points and the reason.

Appendix

Preparation method of Elendt M4 and M7 medium

○ Stock solution of trace elements

Prepare each stock solution (I) of trace elements using deionized water, etc. Prepare the stock solution (II) by blending the certain amount of each solution (I).

Stock solution (I) Substance name	Amount added to dilution water mg/L	Concentration (relationship with M4 medium)	Stock solution (II) Prepare by adding stock solution (I) of the below-listed amount to dilution water. ml/L	
			M4	M7
H_3BO_3	57,190	20,000 times	1. 0	0.25
$MnCl_2 \cdot 4H_2O$	7,210	20,000 times	1. 0	0.25
LiCl	6,120	20,000 times	1. 0	0.25
RbCl	1,420	20,000 times	1. 0	0.25
$SrCl_2 \cdot 6H_2O$	3,040	20,000 times	1. 0	0.25
NaBr	320	20,000 times	1. 0	0.25
$Na_2MoO_4 \cdot 2H_2O$	1,260	20,000 times	1. 0	0.25
$CuCl_2 \cdot 2H_2O$	335	20,000 times	1. 0	0.25
$ZnCl_2$	260	20,000 times	1. 0	1. 0
$CoCl_2 \cdot 6H_2O$	200	20,000 times	1. 0	1. 0
KI	65	20,000 times	1. 0	1. 0
Na_2SeO_3	43. 8	20,000 times	1. 0	1. 0
NH_4VO_3	115	20,000 times	1. 0	1. 0
$Na_2EDTA \cdot 2H_2O$	5,000	2,000 times	-	-
$FeSO_4 \cdot 7H_2O$	1,991	2,000 times	-	-
Na_2EDTA solution and $FeSO_4$ solution are initially prepared separately, poured together just prior to autoclaving.This gives:				
21Fe-EDTA solution		1,000 times	20.0	5. 0

○ M4 medium and M7 medium

Prepare M4 and M7 medium using the stock solution (II), macro nutrient stock solutions and vitamin stock solutions.

	Amount added to dilution water Mg/L	Concentration (relationship with M4 medium)	Added amount of stock solution to prepare medium ml/L	
			M4	M7
Stock solution (II)		20 times	50	50
Macro nutrient stock solutions				
$CaCl_2 \cdot H_2O$	293,800	1,000 times	1. 0	1.0
$MgSO_4 \cdot 7H_2O$	246,600	2,000 times	0.5	0.5
KCl	58,000	10,000 times	0.1	0.1
$NaHCO_3$	64,800	1,000 times	1. 0	1.0
$Na_2SiO_3 \cdot 9H_2O$	50,000	5,000 times	0.2	0.2

$NaNO_3$	2,740	10,000 times	0.1	0.1
KH_2PO_4	1,430	10,000 times	0.1	0.1
K_2HPO_4	1,840	10,000 times	0.1	0.1
Vitamin stock solution	-	10,000 times	0.1	0.1
Prepare vitamin stock solutions by adding 3 vitamins listed below to 1L deionized water. Divide vitamin stock solutions into small portions and preserve in the refrigerator. Add them to medium just prior to use.				
	mg/L			
Thiamine hydrochloride	750	10,000 times		
Cyanocobalamine(B12)	10	10,000 times		
Biotine	7.5	10,000 times		

Notes: In order to prevent from salt precipitation while preparing medium, add a certain amount of stock solution to about 500-800ml deionized water and then fill it up to 1L.

Test on effects of coexistent organic substances on fish acute toxicity / *Daphnia spp* acute immobilization (2-7-3)

1. Summary of studies

To evaluate effects of agricultural chemicals on the actual environment more properly, this study is positioned as the test to examine the toxicity reduction rate of agricultural chemicals against organisms in organic substance-containing water. When the toxicity of an agricultural chemical is expected to decrease due to organic substances, conduct Fish acute toxicity studies or Dalphnia species acute immobilization studies in humic acid with its concentration gradually increased. Divide $L(E)C_{50}$ at TOC concentration 1.5mg/L, calculated based on each TOC concentration and correspondent $L(E)C_{50}$, by $L(E)C_{50}$ in clear water, and obtain the toxicity decreasing coefficient.

2. Test organisms

(1) Organism species

① Select the test organism species which is more sensitive to the test substance from either *Oryzias latipes* or *Daphnia magna*. Daphnia species other than *Daphnia magna* are not acceptable. Specify the source, the breeding method, etc. of organisms to be employed in the test.

② Preferably, conduct the test using the standard substance to confirm reproducibility of the test. Conduct the test using the standard substance for each test using the test substance or for each period (at least twice a year). PCP-Na (pentachlorosodium) and copper(II) sulfate may be employed as the standard substance for fishes and PCP-Na (pentachlorosodium) and potassium dichromate (chromium(Ⅵ); Cr^{6+}) may be employed for daphnia species. (HA concentration of the test standard substance herein corresponds to 0mg/L.)

(2) Acclimatization

Comply with Fish acute toxicity studies.

3. Setting of concentration areas

(1) Preliminary test

Conduct the preliminary test to determine the test concentration range when the toxicity of the test substance in the presence of HA is unclear. In principle, an organic solubilizing agent is not applicable in this test due to its effect on TOC concentration and possible effects of organic substances on toxicity decreasing. However, when less soluble agricultural chemicals are hard to test without an organic solubilizing agent, the minimal amount is acceptable. In this case, consider the necessity of an organic solubilizing agent.

(2) Main test

Set test concentration areas not including HA and test concentration areas including 2.5, 5.0 and 10mg/L HA, and conduct each test for at least five concentration areas. In principle, the separation factor shall be within the range of 1.3-2.2. Apply the same separation factor for each test concentration area of HA.

4. Environmental conditions

(1) Light

Light intensity or quality is not particularly specified herein. Light conditions commonly-used in laboratories are applicable.

(2) Dilution water

① Preferably, use water with the total hardness 10-250mg $CaCO_3$/L and pH 6.0-8.5.

② When using reconstituted water, prepare it using a special or analytical grade reagent and using distilled water or deionized water with conductivity no more than $10\mu Scm^{-1}$.

③ When using tap water and natural water as dilution water, describe the source and all treatment method. When using reconstituted water, describe the composition.

5. Observation and measurement

(1) Test substance concentration

The test substance concentration in the test solution is not required to be measured in this test.

However, when setting the test concentration around the solubility in water, measure the test substance concentration in the test stock solution as appropriate.

(2) Measurement of environmental conditions

① When using dechlorinated tap water or natural water as dilution water, preferably conduct water quality inspection with reference to the water quality parameters prior to the test. Water quality inspection may be periodically conducted.

Keep TOC concentration in dilution water lower to prevent from any effects on the test.

② Measurement of TOC

a. Review the analysis method for measurement of TOC in the sample prior to the test. Confirm the accuracy by three-point sampling of dilution water used in the test, adding the known amount of dissolved organic carbon and analyzing.

b. Conduct measurement of TOC for each HA concentration area in each concentration area and the control prior to applying the test substance and test organisms.

c. When using plural containers for each concentration area, conduct measurement after blending the same amount of the test solution collected from each container.

d. When setting the test concentration areas by preparing a large amount of dilution water of each HA concentration, TOC of this dilution water may be measured.

6. Result assay method

(1) When calculating $L(E)C_{50}$, use common methods such as Probit method, Moving average method, Binomial method and Doudoroff *et al.* method.

(2) Calculate $L(E)C_{50}$ at TOC 1.5mg/L (average TOC of main rivers in Japan).

7. Items to be reported

(1) Describe the following items concerning the test method.

① Detailed chemical analysis method of TOC (device type, repeat accuracy, etc.)

② Exposure conditions

Exposure method (static, semi-static, etc.); test nominal concentration and the separation factor (and summary of the preliminary test); preparation method of test solution; exposure period

③ Environmental conditions

Dilution water; test containers; content density; test solution amount; water temperature; light

8. Literature

Daphnia was added with reference to the US EPA OPPTS 850.1085 "Fish Acute Toxicity Mitigated by Humic Acid".

Freshwater shrimp acute toxicity test (2-7-4)

1. Test organisms

(1) Organism species

① Specify the supply source and breeding method of the test organisms.

② The test organisms shall consist of the organisms which are almost the same in age and size obtained from the same source or cultured group.

③ When using other species than *Neocaridina denticulata* or *Paratya compressa improvisa*, confirm that the test result would be equivalent to the one using the aforementioned species by conducting the comparison test or referring to the known findings, the preliminary test results and/or the test results of the standard substance, and describe the adequacy in the report.

④ Confirm LC50 with the standard substance in compliance with Dalphnia species acute immobilization studies.

(2) Acclimatization

When the supply source of preservation water is different from that of dilution water, take about 48 hours for the test organisms to gradually adapt to dilution water. Subsequently, conduct acclimatization under the environmental conditions of the test for at least 7 days prior to the test day.

Water temperature may fluctuate within 2 degrees C a day. Take care of water quality and feeding amount to prevent from stress or mortality during acclimatization.

(3) Handling test organisms

Prevent from touching the test organisms as far as possible. Handle quickly with the maximum care not to stress them out.

Carefully observe during preservation and acclimatization not to miss any signs of stress and/or mortality.

2. Exposure

(1) Introduce the test organisms into the test container after addition of the test substance.

(2) Cover the test container loosely with a lid to reduce loss of the test solution due to evaporation.

3. Setting test concentration areas

Comply with Fish acute toxicity studies.

4. Preparation of test solution

Comply with Fish acute toxicity studies.

5. Environmental conditions

(1) Housing density

① The number of the test organisms housed in the test container may not be excessive to prevent effects on the test results.

② Set the dissolved oxygen concentration at no less than 60% of the air saturation value.
③ In flow-through studies, the housing density depends on a flow rate of dilution water.

(2) Light
Light intensity or quality is not particularly specified herein. The light conditions usually employed in laboratories are applicable.

(3) Dilution water
① When using dechlorinated tap water or natural water as dilution water, preferably inspect dilution water quality prior to the test with reference to the water quality parameters. Water quality may be periodically inspected.
② When using reconstituted water, use a special or analytical grade reagent and use distilled water or deionized water with conductivity no more than $10\mu^{-1}$Scm for preparation.
③ When using tap water and natural water as dilution water, describe the supply source and pretreatment method. When using reconstituted water, describe the composition.

(4) pH
Even if pH fluctuates due to addition of the test substance, do not adjust the pH of the test solution.

(5) Dissolved oxygen concentration
When conducting gradual aeration as appropriate, the marginal aeration is allowable to prevent any effects on the test.

6. Observation and measurement

(1) Observation
① Report whenever observing any abnormal behavior and appearance as well as mortality of the test organisms.
② Observe conditions of the test solution and record the emergence of a membrane on the water surface, sedimentation and substances adherent to the test container.

(2) Measurement of test substance concentration
Comply with Fish acute toxicity studies.

(3) Measurement of environmental conditions
① Measure water temperature, dissolved oxygen concentration and pH of the test solution in at least one replicate among all concentration areas.
② Other items shall comply with Fish acute toxicity studies.

7. Result assay method

(1) In principle, calculate LC_{50} based on the average measured concentration. When fluctuation against the nominal concentration is less than ±20%, calculation based on the nominal concentration is applicable.

(2) Other items shall comply with Fish acute toxicity studies.

8. Items to be reported

Comply with Fish acute toxicity studies.

9. Literature

The Japanese Society of Environmental Toxicology: Ecology Effect Study Handbook: Asakura Publishing, 2003, pp.100-106

Amphipoda acute toxicity test (2-7-5)

1. Test organisms

(1) Organism species

① When using other species than *Gammarus fasciatus*, *G. pseudolimnaeus*, *G. lacustris* and *Hyalella azteca*, confirm that the test result would be equivalent to the one using the aforementioned species by conducting the comparison test or by referring to the known findings, results of the preliminary test and the test of the reference substance, and describe the adequacy in the report.

② Others shall comply with Freshwater shrimp acute toxicity studies.

(2) Acclimatization

Comply with Freshwater shrimp acute toxicity studies.

(3) Handling method

Comply with Freshwater shrimp acute toxicity studies.

(4) Feeding

Feed with deciduous leaves (e.g., maple trees, poplar and birch) after dipped in running water for at least 30 days until matured. Provide leaves sufficient enough to cover the bottom of the breeding container with multiple layers. If eaten out, feed with more leaves.

2. Exposure

Comply with Freshwater shrimp acute toxicity studies.

3. Setting test concentration areas

Comply with Fish acute toxicity studies.

4. Preparation of test solution

Comply with Fish acute toxicity studies.

5. Environmental conditions

Comply with Freshwater shrimp acute toxicity studies.

6. Observation and measurement

Comply with Freshwater shrimp acute toxicity studies.

7. Result assay method

Comply with Freshwater shrimp acute toxicity studies.

8. Items to be reported

Comply with Fish acute toxicity studies.

9. Literature

The Japanese Society of Environmental Toxicology: Ecology Effect Study Handbook: Asakura Publishing, 2003, pp.107-109

Chironomus sp., acute immobilization test (2-7-6)

1. Test organisms

(1) Use test organisms whose history (e.g., the supply source) is clarified. Particularly, it is difficult to identify the species using only chironomid larva so that identification shall be conducted by breeding larva until eclosion and matching it to imago. It is best to obtain the organism species subcultured and already identified.

(2) Breeding chironomid
Since first instar larvae are used in the study, the breeding is performed in the following procedures:
① Place a egg mass in a glass container (petri dish) filled with test water and wait for them to hatch in the same conditions as in the study.
② After hatching, the larvae are given dry yeast or powdered feed for fish until being subjected to the experiment.

(3) Confirm LC_{50} with the standard substance in compliance with Dalphnia species acute immobilization tests.

2. Setting test concentration areas

(1) In principle, the separation factor shall be within the range of 1.3-2.2.

(2) The maximum test concentration is 100 mg/L.

3. Preparation of test solution

Comply with Fish acute toxicity tests.

4. Environmental conditions

(1) Lighting
The light strength is preferably 500-1000 lux. The light quality is not specified.

(2) Dilution water
In principle, the dilution water should be selected according to fish acute toxicity tests, the use of Elendt M4 or M7 is proposed as reconstituted water in OECD Test Guideline 235 *Chironomus* sp., Acute Immobilisation Test (2011).

(3) pH
Regardless of pH fluctuation due to addition of the test substance, do not adjust the pH of the test solution.

(4) Other items shall comply with Fish acute toxicity tests.

5. Observation and measurement

(1) Observation of general conditions of test organisms

Record whenever observing the difference from the control such as decreased activities and atrophy of chironomid larva.

(2) Measurement of test substance concentration and environmental conditions

Comply with Fish acute toxicity tests.

6. Result assay method

Comply with Fish acute toxicity tests.

7. Items to be reported

Comply with Fish acute toxicity tests.

Algae growth inhibition test (2-7-7)

1. Test organisms

(1) Organism species

① For TGAI, studies using *Pseudokirchneriella subcapitata* (former name: *Selenastrum capricornutum*) are necessary. For formulation products, studies using *Pseudokirchneriella subcapitata* are recommended. When using other species than *Pseudokirchneriella subcapitata*, confirm that the test result would be equivalent to the one using the aforementioned species by conducting the comparison test or referring to the known findings, the results of the preliminary test results and/or the standard substance. When using other species than *Pseudokirchneriella subcapitata*, describe the adequacy in the report. Clarify the supply source of the test algae.

② Preferably, conduct the test using the standard substance to confirm reproducibility of the test. Conduct the test using the standard substance for each test substance or for each period (at least twice a year). PCP-Na (pentachlorophenol sodium) and potassium dichromate (chromium(Ⅵ); Cr^{6+}) are applicable as the standard substance. However, note that pH would fluctuate the test of PCP-Na. Waste leftover solutions for preparation of chemicals and used chemicals after proper treatment. The treatment methods of potassium dichromate include reduced-chemicals sedimentation method and ion exchange, etc.

Describe EC_{50} with the standard substance used along with background data (average±standard deviation) in the test report.

2. Setting test concentration areas

Comply with Fish acute toxicity tests.

3. Preparation method of test medium

(1) Commonly-used solubilizing agents today include N,N-dimethlyformamide, triethyleneglycol, acetone, ethanol, methanol and hydrogenated castor oil, etc.

(2) Two or more kinds of solubilizing agents may be combined. In this case, the solubilizing

agent concentration in the test solution shall be constant for all the test concentration areas in principle and preferably no more than 100mg/L (or 0.1ml/L).

4. Environmental conditions

(1) Light

Continuous uniform illumination should be provided. Where a recommended strain of *Pseudokirchneriella subcapitata* is used, the light intensity should be preferably in the range of 60-120 μE/m^2/s (4440-8880 lux) measured in a wavelength range of 400-700nm near the solution surface.

(2) Culture medium

① Medium type

Preferably, use OECD medium or AAP (AGP) medium.

a. OECD medium

NH_4Cl	15	mg/L
$MgCl_2 \cdot 6H_2O$	12	mg/L
$CaCl_2 \cdot 2H_2O$	18	mg/L
$MgSO_4 \cdot 7H_2O$	15	mg/L
KH_2PO_4	1.6	mg/L
$FeCl_3 \cdot 6H_2O$	0.064	mg/L
$Na_2EDTA \cdot 2H_2O$	0.1	mg/L
H_2BO_3	0.185	mg/L
$MnCl_2 \cdot 4H_2O$	0.415	mg/L
$ZnCl_2$	3	μg/L
$CoCl_2 \cdot 6H_2O$	1.5	μg/L
$CuCl_2 \cdot 2H_2O$	0.01	μg/L
$Na_2MoO_4 \cdot 2H_2O$	7	μg/L
$NaHCO_3$	50	mg/L

The pH of this medium after equilibration with air is approximately 8.1.

b. AAP(AGP) medium

$NaNO_3$	25.5	mg/L
K_2HPO_4	1.044	mg/L
$MgCl_2 \cdot 6H_2O$	12.16	mg/L
$MgSO_4 \cdot 7H_2O$	14.6	mg/L
$CaCl_2 \cdot 2H_2O$	4.41	mg/L
$NaHCO_3$	15	mg/L
H_3BO_3	0.186	mg/L
$MnCl_2 \cdot 4H_2O$	0.415	mg/L
$ZnCl_2$	3. 27	μg/L
$CoCl_2 \cdot 6H_2O$	1.43	μg/L
$CuCl_2 \cdot 2H_2O$	0.012	μg/L
$Na_2MoO_4 \cdot 2H_2O$	7.26	μg/L
$FeCl_3 \cdot 6H_2O$	160	μg/L
$Na_2EDTA \cdot 2H_2O$	0.30	mg/L

The pH of this medium after equilibration with air is approximately 7.5. Adjust pH to 7.5±0.1 with dilute hydrochloric acid or sodium hydroxide aqueous solution if necessary.

When using a reagent of a different water content, the amount should be converted on an active ingredient basis.

When using other medium, comply with the maximum value of essential ingredients listed below.

P	≦	0.7	mg/L
N	≦	10	mg/L
Chelating agent	≦	10^{-3}	mmol/L
Hardness (Ca ＋ Mg)	≦	0.6	mmol/L

5. Observation and measurement

(1) Measurement of biomass

① Since biomass is difficult to determine by directly measuring the dry weight of algae in the test solution, it can be substituted with cell concentrations measured by particle counter or hemocytometer, or with fluorometric, spectrophotometric or turbidimetric measurements as an alternative parameter. However, the relevance of the alternative parameter with biomass should be confirmed.

② Morphologically altered cells may be included in the measurement of cell concentration measurement, but should be reported as study findings.

(2) Measurement of test substance concentration

① When using a TGAI as the test substance, measure the concentration in each test concentration area to confirm the test substance concentration in the test solution and obtain information of concentration stability.

② Measure the concentration at least at the commencement and the end of exposure.

③ Preferably, the test substance concentration at the commencement of exposure is at least 80% of the nominal concentration.

④ Collect samples for measurement from the intermediate layer of the test solution. Even if the test substance partially precipitates or floats on the surface layer, refrain from stirring in principle.

⑤ When using a formulation in the test, measurement of the test substance concentration is not required.

⑥ For measurement of the dissolved test substance concentration, analyze the sample after conducting centrifugal separation at a low g-force and removing algae.

(3) Measurement of environmental conditions

Measure water temperature and pH of the test solution in each experimental area, each test concentration area and the control. Conduct measurement at least at the commencement and the end of exposure. Preferably, conduct measurement every 24 hours to confirm the fluctuation range.

6. Result assay method

(1) Exposure concentration for calculating concentration-inhibition rate

When using a TGAI as the test substance and the test substance concentration fluctuation throughout the experimental term is less than ±20% of the nominal concentration or the measured concentration at the commencement of exposure, the nominal concentration or the measured concentration at the commencement of exposure is respectively applicable to the result assay.

The calculation method of the average measured concentration shall comply with Fish acute toxicity studies.

(2) Calculation method of growth rate and growth inhibition rate

① Calculation method of growth rate

The growth rate in the exponentially growing culture ($\mu_{i\text{-}n}$) is calculated according to the following equation.

$$\mu_{i\text{-}n} = \frac{\ln X_n - \ln X_i}{t_n - t_i}$$

$\mu_{i\text{-}n}$: average growth rate (/day) from i to n

X_i: biomass at time t_i. For the biomass at the start of the test (t_0), use nominal values.

X_n: biomass at time t_n

t_i: time of the i^{th} biomass measurement after the start of the test (day)

t_n: time of the n^{th} biomass measurement after the start of the test (day)

l_n: natural logarithm

Alternatively, the average growth rate during the exposure period can be obtained from the slope of the regression line drawn by plotting l_nX against time.

② Calculation method of growth inhibition rate

Growth inhibition rate for the replicates of each test substance concentration should be a value (I_μ) calculated from the difference between the mean (μ_c) of the average growth rates of replicated controls during the exposure period and the mean growth rate (μ_t) for the replicates of each test substance concentration during the exposure period.

$$I_\mu = \frac{\mu_c - \mu_t}{\mu_c} \times 100\ (\%)$$

When solubilizing agents are used to prepare the test solutions, the solubilizing agents controls rather than the controls without solubilizing agents should be used in calculation of growth inhibition rate.

③ Preparation of the concentration -growth inhibition rate curve

The concentration-growth inhibition rate curve should be drawn on a semilogarithmic or semilogarithmic normal probability paper. On the graph should be plotted Iμ values for individual replicates of each test substance concentration.

(3) Calculation of 50% growth inhibition concentration

① Grasp the relationship between the exposure concentrations and growth inhibition rates on the graph created according to (2) ③, and calculate the toxicity values by regression analysis. In this case, suitable data transformation methods may be used, such as Probit, Logit, and Weibull transformations.

② 50% growth inhibition concentration is abbreviated as ErC50 to indicate that mean growth rates are used in the calculation.

7. Items to be reported

(1) For test organisms, incubation method at preculture, etc. should also be described.

(2) For study methods, the following should be described.

① Exposure conditions

Initial biomass; exposure method (shaken culturing); test nominal concentration and separation factor (and summary of the preliminary test); preparation method of test medium (the type and concentration of a solubilizing agent when using); exposure period, etc.

② Environmental conditions
Medium; incubation method; test container and device; water temperature; lighting, etc.
③ Observation and measurement items, etc.
Observation items and method; measurement method of test substance concentration (when using a TGAI as the test substance); measurement method of water temperature and pH; result assay method, etc.

(3) Test results
① Observed effects include morphological transformation.
② The test solution conditions concerning other items means presence or absence of precipitation and sedimentation, etc.

(4) When modifying the test method, report the details of modification and the reason.

Test on toxicity on beneficial organisms other than aquatic animals and plants (2-8-1～4)

Bee toxicity test (2-8-1)

For the acute toxicity study, an example of the test method of acute oral/contact toxicity studies and field toxicity studies in compliance with the OECD test guideline is given below. Alternatively, field toxicity studies are applicable in place of acute toxicity studies.

1. Acute oral toxicity study

The principle of this test complies with the OECD test guideline 213.

(1) Test method
① Test bees
Use young worker bees of *Apis mellifera*. Select the ones whose history (e.g., the supply source and breeding method) is clarified. Collect test bees in the morning of the treatment day or in the early evening of the day before the treatment day. Control them under the same conditions as the experimental term until the test commences to acclimatize them. Give them nothing to eat for two hours prior to administration.
② Experimental area
Set at least five dose areas in geometrical progression, the not-treated control including no test substance, and the area of the standard substance (whose toxicity is known, e.g., dimethoate) including at least three dose.
However, in the preliminary test to confirm low toxicity, provide one dose area and the non-treated control. Dose of the administration area shall be 100μg/organism.
Provide at least three areas each of which includes at least 10 organisms.
③ Exposure and breeding method
Dissolve or disperse the test substance in 50% (W/V) sucrose solution, and in principle, feed with 100-200μl per 10 test bees for four hours (no more than six hours). Provide 50% sucrose solution after the test substance administration. Control the breeding conditions at 25±2 degrees C, 50-70% humidity and complete darkness.
④ Experimental term

Record each number of alive, dead and abnormal bees 4, 24 and 48 hours after the commencement of exposure.
However, if the mortality rate between the 24-48th hour excesses 10%, extend up to the 96th hour. Conduct measurement of the consumption of the test substance solution during exposure.

⑤ Others
Details of other test conditions shall comply with the OECD test guideline 213.

(2) Result report
In principle, describe the items listed below.

① Testing agency and the person in charge (section)
② Information of the test substance
When using a TGAI: general name, chemical name, structural formula, purity and lot number
When using a formulation: type, active ingredient content, and lot number
③ Experimental area constitution
④ Test method
⑤ Test results
a. LD_{50} (μg/organism) at each observation time
b. Calculation method of LD_{50}
c. Cumulative mortality rate in each experimental area at each observation time
d. Details of abnormal behaviors observed
e. Other data and information required for result analysis

2. Acute contact toxicity studies

The principle of this study complies with the OECD test guideline 214.

(1) Test method

① Test bees
Comply with Acute oral toxicity studies.
② Experimental area
Comply with Acute oral toxicity studies.
③ Exposure and breeding method
Dissolve the test substance in water to which an organic solvent or a wetting agent is added. Locally apply 1μl test substance to the backside of the chest of each anesthetized test bee. Control the breeding conditions at 25±2 degrees C, humidity about 50-70% and complete darkness.
④ Experimental term
Comply with Acute oral toxicity studies.
⑤ Others
Details of other test conditions shall comply with the OECD test guideline 214.

(2) Result report
Comply with Acute oral toxicity studies.

3. Field toxicity studies

(1) Test method
Use typical crops which honeybees visit. Apply the agricultural chemical once at the practical maximum dose (concentration) at the time of flowering to research effects on honeybees (e.g., mortality and abnormal behavior) for a certain period.

(2) Result report
In principle, describe the items listed below.

① Testing agency and the person in charge (section)
② Information of the test substance

Type, active ingredient content, lot number

③ Test method

④ Test results

a. Properly organize each item such as mortality and abnormal behaviors.

b. Consideration

Consider the content, degree and period of the effects.

4. Publications

(1) EPPO (1992).Guideline on Test Methods for Evaluation the Side-Effects of Plant Protection Products on Honeybees (No.170).OEPP/EPPO Bulletin 22,203-215.

(2) Harrison,E.G.(1993).Proceedings of the Fifth International Symposium on the Hazards of Pesticides to Bees, October 26-28, 1993, Plant Protection Service, Wageningen, The Netherlands. Report IUBBS,14pp + Appendices 180pp.

(3) SETAC81995).Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticide. Edited by Dr. Mark R.Lynch. Published by SETAC-Europe, Belgium. March 1995.

(4) Stute, K. (1991). Auswirkungen von Pflanzenschutzmitteln auf die Honigbiene. Richtlinien fur die Prufung von Pflanzenschutzmitteln im Zulassungsverfahren, Teil VI, 23 - 1, Biologische Bundesanstalt fur Land - und Forstwirtschaft (BBA), Braunschweig, Germany.

(5) US-EPA(1995). Honey Bee Acute Contact Toxicity Test (OPPTS 850.3020). Ecological Effects Test Guidelines. EPA 712-C-95-147, Washington DC, United States of America.

(6) EPPO/Council of Europe. (1993). Decision-Making Scheme for the Environmental Risk Assessment of Plant Protection Products - Honeybees. EPPO bulletin, vol. 23, No.1, 151-165. March 1993.

(7) Gough, H.J, McIndoe, E.C, Lewis, G.B. (1994). The use of dimethoate as a reference compound in laboratory acute toxicity test on honey bees (Apis mellifera L.) 1981-1992. Journal of Apicultural Research 22, 119-125.

(8) ICPBR Validation Exercise on the Use of Dimethoate as a Toxic Reference Substance in Toxicity Test on Honeybees (in preparation).

(9) Litchfield, J.T. and Wilcoxon, f. (1949). A simplified method of evaluating dose-effect experiments. Jour. pharmacol. and Exper. Ther., 96, 99-113.

(10) Finney, D.J. (1971). Probit Analysis. 3rd ed., Cambridge, London and New-york.

(11) Abbott, W.S. (1925). A method for computing the efficacy of an insecticide. Jour. Econ. Entomol. 18, 265-267.

(12) U.S.EPA/OPPTS Guideline 850.3030"Toxicity of Residue on Foliage"

(13) EPPO 170 "Cage tests"

Silkworm toxicity test (2-8-2)

An example of the test method for acute oral toxicity studies and remained toxicity studies is given below. Alternatively, remained toxicity studies may be conducted in place of acute toxicity studies.

1. Acute oral toxicity studies

(1) Test method

① Test silkworms: a practical breed

② Experimental area constitution

Provide the non-treated area (same as the treated area except for containing no test substance) as the control.

The treated area must be studied at the practical maximum dose (concentration) of the agricultural chemical.

③ Specimen conditions

After spraying a certain amount on mulberry leaves and air drying, feed silkworms which are bred with a certain method every day during the 4th age period. When using an artificial diet, adjust the contained amount of agricultural chemicals in accordance with the food consumption during the 4th age period.

④ Experimental term

Conduct the test until cocooning.

(2) Result report

In principle, describe the items listed below.

① Testing agency and the person in charge (section)

② Information of the test substance

The type, active ingredient content and lot number, etc.

③ Experimental area constitution

④ Test method

⑤ Test results

a. Mortality
b. Toxic symptom
c. The number of days during the 4-5th age period
d. Survival rate of pupae and cocoon quality
e. Other items which have effects on silkworms
f. Consideration

2. Residual toxicity studies

The purpose of this test is to research the time course of the residual toxicity by feeding silkworms with mulberry leaves sprayed with the test substance.

(1) Test method

① Test silkworm: a practical breed

② Experimental area constitution

Non-treated area: same as the treated area except for containing no test substance

Treated area: at the practical maximum dose (concentration) of the agricultural chemical

③ Specimen conditions

Feed the test silkworms every day during the 4th age period with treated leaves in each area all of which are collected on the first day after the set period passed.

④ Experimental term

Conduct the test until cocooning.

(2) Result report

In principle, describe the items listed below.

① Testing agency and the person in charge (section)
② Information of the test substance
③ Type, active ingredient content, and lot number, etc.
④ Experimental area constitution
⑤ Test method
⑥ Test results
⑦ Number of mortality and decreasing rate of silkworms on day basis
⑧ Toxic symptom
⑨ Uniformity rate of growth

⑩ Number of days during the 4-5th age period
⑪ Survival rate of pupae and cocoon quality
⑫ Indicative number of safety days
⑬ Other items which have effects on silkworms
⑭ Consideration

3. Publications

(1) Test methods of JPPA "Guidelines for efficacy research of agricultural chemicals on silkworms", January 1995, Japan Plant Protection Association

Natural enemy insect, etc. toxicity test (2-8-3)

An example of the test method for the acute toxicity studies and the field test is given below. Alternatively, the field test may be conducted in place of acute toxicity studies.

1. Acute toxicity studies

(1) Test method

① Test insects

In accordance with the applied crops and the employed method, in principle, select at least two orders and three species among predatory insects (Diptera (Episyrphus), Hemiptera (shield bugs), Coleoptera (Ladybird beetles, Poecilus), Neuroptera (Chrysopidae species, etc.)); parasitic bees (Hymenoptera (Aphidius, Trichogramma, Aphelinidae)); and Araneida (Pardosa species) predatory mites (Acarina (Phytoseiidae species, etc.)) as the test insects.

Use the test insects all of which are the closest in day-based age, behaviors, fertility and egg activities as far as possible. Preferably, use the test insects at the developmental period in which insects become sensitive.

② Experimental area constitution

Non-treated area: same as the treated area except for containing no test substance

Treated area: at the practical maximum dose (concentration) of the agricultural chemical

③ Exposure method and breeding conditions

In accordance with the property of the test substance and test insects, select the path to expose the test insects enough to sufficiently contact the test substance.

Breed the test insects under their optimum conditions.

④ Experimental term

Determine the term properly in accordance with the property of the test substance and test insects

(2) Result report

In principle, describe the items listed below.

① Testing agency and the person in charge (section)
② Information of the test substance

General name, chemical name, structural formula, purity, and lot number, etc.

③ Organism species name
④ Experimental area constitution
⑤ Test method
⑥ Test results

a. Mortality rate
b. Abnormal behaviors
c. Research the following items properly in accordance with the property of the test substance and test insects.
   [a] Predation or parasitic property
   [b] Growth conditions (period, body length, etc.)
   [c] Pupation rate (in case of holometabolous insects)
   [d] Oviposition ratio and number
   [e] Survival rate of the next generation (hatch rate)
d. Consideration

2. Field test

(1) Test method

① Test insects

Select the field where the test insects appear. If necessary, release and breed the test insects therein.

② Experimental area constitution

Non-treated area: same as the treated area except for containing no test substance

Treated area: treated with the practical maximum dose (concentration) of the agricultural chemical once

③ Exposure method

Treat the test substance in the field or facility where the typical one of the agricultural crops specified in the registration application was raised with the method specified in the registration application.

④ Experimental term

Set the experimental term properly in accordance with the kind of the test substance and test insects.

⑤ Research method

Use the proper method according to the property such as the kind of the test substance and test insects.

(2) Result report

In principle, describe the items listed below.

① Testing agency and the person in charge (section)

② Information of the test substance

Type, active ingredient content, and lot number, etc.

③ Organism species name

④ Experimental area constitution

⑤ Test method

⑥ Test results

a. Number of each organism species
b. Age of each organism
c. Properly organize each item such as mortality and abnormal behaviors.
d. According to the property of the test substance and the test insects, properly research and organize the properties such as predation, parasitism or reproduction.
e. Consideration

3. Publications

(1) BARRETT, K.L., et al. (1994): Guidance document on regulatory testing procedures for pesticides with non-target arthropods, SETAC-Europe, pp. 51.

(2) HASSAN, S.A. (1985): Standard methods to test the side-effects of pesticides on natural enemies on insects and mites developed by the IOBC/WPRS working group,

"Pesticide and biological organisms", Bull. OEPP/EPPO 15: 214-255.
(3) HASSAN, S.A. (1992): Guidelines for testing the effects of pesticides on beneficial organisms: Description of test methods, IOBC/WPRS Bulletin, 1992/XV/3, pp. 186.
(4) HIRAI Kazuo (1996): Efficacy assessment standard draft of chemical pesticides on non-targeted organisms –Examples in EU-: Plant protection 50: 285-289.
(5) HIRAI Kazuo, MORI Katsuhiko (1997): Present situation and issues of ecotoxicology studies on agricultural chemicals and beneficial organisms: Plant protection 51: 72-73.
(6) HIRAI Kazuo (1999): Natural enemy organisms: Overview/Basic efficacy assessment of chemical pesticides: Plant protection 53: 197-200

Avian toxicity test (2-8-4-1, 2)

Avian acute oral toxicity test (2-8-4-1)

Conduct studies with the test method reference to U.S. EPA 712-C-96-139 April 1996 Ecological Effects Test Guidelines OPPTS 850.2100 Avian Acute Oral Toxicity Test "Public Draft", etc.

Avian dietary toxicity test (2-8-4-2)

Preferably, comply with the OECD test guideline 205 Avian Dietary Toxicity Test, 1984.

The summary of the test method is given below.

1. Test organisms

(1) Organism species

Use one or more species of avian. The species should be selected in accordance with the purpose of the test. Preferably, select the test species based on the experience of breeding or testing under the laboratory conditions. Birds should be in good health and have no apparent deformity. The recommended birds for the test are listed in Table 1. When using other species, adjust the test method to provide the proper test conditions.

Birds listed in Table 1 are easy to breed and available throughout the year. Birds can be purchased or hatched from eggs. When purchasing birds, confirm that they are not infected with diseases as aspergillosis, Newcastle disease and pullorum or they are hatched from the group in which such diseases are not observed.

(2) Growth stage

The birds in all of the experimental areas and the control should be originated from the same group in the known lines. At least when using the chicks 10-17 days old, the difference of the age from one another should be within one day.

(3) Acclimatization

The birds should be acclimated to the facilities and the fundamental diet for at least

seven days. Randomly place the birds in the cage and randomly assign the concentration level to be administrated to the cage. Place the fundamental diet so that the birds may arbitrarily eat.

Investigate the health condition of the group within 72 hours prior to the test. Record the mortality rate and follow the standard listed below.

① If the mortality rate due to health conditions or unknown reasons is no less than 5%: Abandon the group completely.

② The mortality rate of the group is no more than 5%: The group may be tested.

(4) Test bird number

Each group consists of at least 10 birds.

2. Test method

(1) Experimental period

Feed the birds for five days with the diet containing the test substance prepared at a sequence of concentrations. Feed the birds with the fundamental diet containing no test substance for at least three days from the 6^{th} day.

If some birds die on the 7th or 8th day, the toxic symptom remains on the 8th day or recovery is not clearly confirmed, continue the test until no mortality is recognized for two successive days, recovery is confirmed or until the 21st day from the commencement of the test.

(2) Preparation of diet

Prepare the diet containing the test substance at least five different concentrations and prepare the non-treated diet. The separation factor at each concentration level shall be at no more than 2.0. To set the employed concentration, conduct the toxicity range finding test.

In principle, the maximum test concentration shall be 5000ppm. When no mortality or toxicity effect is recognized at this concentration, the test at five concentration levels is not required.

Prepare the diet containing the required amount of the test substance by uniformly mixing the proper amount of the test substance and the prescribed fundamental diet for chicks. The standard to select the method of mixing is that the test substance may be uniformly dispersed in the prey. If necessary, a low-toxicity solubilizing agent is applicable to help uniform dispersion. A solubilizing agent should be no more than 2% of the diet by weight. When using a solubilizing agent, provide the control of the solubilizing agent.

Water, corn oil or other solubilizing agents are applicable if clearly proved not to change toxicity of the test substance.

Place the diet containing the test substance or the non-treated diet so that the birds can arbitrarily eat.

Refrain from preventive medicines or other medicines as far as possible. Report the details when using them.

3. Environmental conditions

The environmental conditions particular to the species are given in Table 1.

Maintain the general environmental conditions listed below and avoid any environmental change which has substantial effects on bird activities.

(1) Clean water can be arbitrarily taken.

(2) Set the specific period of 12-16 hours of light per day.

(3) Place up to 5-10 birds in one cage. In case of pigeons, place one pigeon in one cage.

(4) Ventilation should be sufficiently conducted.

4. Observation and measurement

Observe at least the items listed below during the experimental period.

(1) Toxic symptoms and other abnormal behaviors: Observe twice on the 1st day of treatment, and once every day on the subsequent days.

(2) Mortality rate: Observe twice on the 1st day of treatment, and once every day on the subsequent days.

(3) Body weight: Observe on the 0th, 5th and 8th day of treatment and on the final day of the test (if the experimental term is over 8 days).

(4) Food consumption: observe on the 0-5th day, the 5-8th days and the 8th–final days (if extending the test).

5. Result assay

Median lethal concentration (LC_{50}) may be determined by Probit analysis, other suitable statistical methods or using a graph (see Publications 7, 8 and 9). Calculate the 95% confidence limit with a proper method if sufficient data is available, examine statistical nonuniformity, to ascertain the validity of the data.

When the Probit analysis using data obtained from the test for the separation factor 2 or less is not suitable for calculation of LC_{50} (i.e., when no or all test organisms was dead in almost all cases), obtain LC_{50} using the highest concentration which results in no mortality, the lowest concentration which results in 100% mortality and the concentration which results in partial mortality (see references 9, 10 and 11).

When the mortality rate at 5000ppm is no more than 50% (of the highest recommended treatment concentration) and LC_{50} cannot be calculated, report that LC_{50} is greater than 5000ppm and concurrently report the no effect level.

6. Report

In principle, the report should include the items listed below.

(1) Information of the test substance

General name, chemical name, structural formula, purity and lot number, etc.

(2) Test organisms

Scientific name and strain of the species; and day-based age of birds on the 1st day of the test

(3) Experimental conditions

① Cage conditions (form, size and material; temperature; estimated humidity; lighting hours and illuminance)
② Fundamental diet (supply source; composition; analysis results by manufacturers (proteins, carbon hydrates, fat, calcium, phosphorous, other additives and solubilizing agents)
③ Test prey (preparation method; number of concentrations; nominal and measured test substance concentration (when measured) in the diet; measurement method of concentration; frequency of mixing and renewal; solubilizing agents (when using); stock conditions and administration method)
④ Methods of acclimatization operation and random placement of birds in the cage
⑤ Number of cages for each concentration and the control; number of birds per cage
⑥ Frequency, period and method of observation
⑦ Substance name and preparation method of test diet when using the standard substance

(4) Test results

① Number of dead birds at each treatment level and in the control group

② Body weight (average weight of alive birds in each cage at the commencement of the test, at the end of exposure and at the end of the test; individual weight of all dead birds throughout the experimental period)

③ Toxic symptoms (cramps or decreased activities) and other abnormal behaviors (abnormal interactions with other birds)
Describe the date of commencement of the test; the experimental period; the degree of toxic symptoms (including mortality); and the number of birds affected by each test concentration and the control diet for each day throughout the experimental period.

④ Food consumption (Food consumption per cage during the exposure period and the subsequent recovery period should be measured with weigh-back method.)

⑤ Results of toxicity range finding test (only when conducting this test)

⑥ LC_{50}
Describe the 95% confidence limit; the gradient of the concentration-mortality rate curve; results of the compatibility test and the χ^2 examination; the maximum concentration which results in no mortality and the minimum concentration which results in 100% mortality; the employed statistical method or the references.

7. Validity of the test

(1) The mortality rate in the control should not exceed 10% at the end of the test.

(2) Demonstrate that the test substance concentration in the diet is sufficiently maintained (at least 80% of the nominal concentration) throughout the first five days of the experimental period.

(3) At the lowest treatment concentration, mortality or other obvious toxicity effects due to the test substance may not occur.

Table 1 Recommended bird species and environmental conditions

Recommended species		Recommended conditions			
		temperature (degrees C)	humidity (%)	age (days)	space (cm^2/bird)
Mallard *Anas platyrhynchos*			60 - 85	10 - 17	600
Age	0 - 7 days	32 - 35			
	8 - 14 days	28 - 32			
	>14 days	22 - 28			
Bobwhite quail *Colinus virginianus*					
Age	0 - 7 days	35 - 38	50 - 75	10 - 17	300
	8 - 14 days	30 - 32			
	>14 days	25 - 28			
Pigeon *Columba livia*					
Age	>35 days	18 - 22	50 - 75	56 - 70	
	2500*				
Japanese quail *Coturnix coturnix japonica*					

Age	0 - 7 days	35 - 38	50 - 75	10 - 17	300
	8 - 14 days	30 - 32			
	>14 days	25 - 28			

Ring-necked pheasant *Phasianus colchicus*

Age	0 - 7 days	32 – 35	50-75	10-17	600
	8 - 14 days	28 - 32			
	>14 days	22 - 28			

Red-legged partridge *Alectoris rufa*

Age	0 - 7 days	35 - 38	50 - 75	10 - 17	450
	8 - 14 days	30 - 32			
	>14 days	25 - 28			

* Place pigeons separately.

8. Literature

(1) U.S.EPA: Registration of Pesticides in the United States-Proposed Guidelines *Federal Register 43*, 123 (July 10, 1978).
(2) Toxic Sustances Control Act, Section 4: Five-day Dietary Toxicity Test Standard for Mallard and Bobwhite Office of Toxic Substances, U.S.EPA, Washinggton, D.C.
(3) Pesticides Sfety Precautions Scheme, Working Document D S: Evaluating the Acute Oral and Short-Terme Cumulative Oral toxicity of Pesticides to Birds, Tolworth Laboratory, Ministry of Agriculture, Fisheries and Food, U.K (1979).
(4) Protocols for Sub-acute Toxicity Test (LC50-8 days) in Quail and Mallards, Central Institute for Nutrition and Food Research, TNO, The Netherlands.
(5) E.F.Hill, R.G.Heath, J.W. Spann and J.D. Williams: Lethal Dietary Toxicities of Environmcntal Polluants to Birds, U.S.Fish and Widlife Service, Special Scientific Report-Wildlife N 191, Washington, D.C. (1975)
(6) National Research Council: Coturnix.Standards and Guidelines for the Breeding, Care, and Management of Laboratory Animals, U.S. National Academy of Sciences, Washington, D.C. (1969).
(7) D.J. Finney, Probit Analysis, 3rd ed, Cambridge University Press, London (1971)
(8) J.J. Litchfield and F.Wilcoxon, *J.Pharmacol.Exper.Ther.96*, 99-113 (1949).
(9) C.E.Stephan, in Aquatic Toxicology and Hazard Evaluation (edited by F.L.Mayer and J.L.Hamelink), ASTM STP 634, pp. 65-84, American Society for Testing and Materials (1977).
(10) W.R.Thompson, *Bacleriological Review 11*, 115-145 (1974).
(11) C.S.Weil, *Biometrics 8*, 249-263 (1952)

Test on the properties, stability, degradability, etc. of active ingredients (2-9-1 ～ 17)

Preferably, use a high-purity substance as the test substance in this study. Thus, use a preparation purified as far as possible. When using a low-purity substance, specify the reason (e.g., difficulties in purification). Describe the purity of the test substance employed in each test in the report.

Color test (2-9-1)

1. Natural light is acceptable.

2. Record the observation conditions (e.g., temperature and lighting conditions).

3. In principle, indicate a color tone in the term defined in the JIS.

Test on physical state of the substance (2-9-2)

Record the observation conditions (e.g., temperature and lighting conditions).

Odor test (2-9-3)

1. This study is not required if it would be harmful to observer's health. However, specify the reason.

2. Record the observation conditions (e.g., temperature).

3. For example, odor may be expressed as: "stimulant odor", "aromatic odor", "sulfuric odor", "ether odor", "phenol odor", "aldehyde odor", "amine odor", "garlic-like odor", "specific odor" and "non-odor" along with strong/weak expressions such as "strong" and "subtle".

Spectrum test (2-9-4)

1. Record the employed measurement device (the manufacturer and the type) and the measurement conditions (e.g., the general conditions including temperature, solvent type and test substance concentration; each specific measurement condition including IR sample preparation, MS introduction condition, ionization condition, NMR measured nucleus and standard substance).

2. The wavelength of UV/VIS shall be measured up to almost 220-400nm. When using a colored substance, the visible region shall be measured. When difficult to clearly indicate all the peak tops at the same intensity due to some intensity-different absorption, preferably attach an enlarged/reduced chart. For components dissociated depending on pH, conduct measurement of spectra of dissociation and non-dissociation.
   When rapid decomposition is observed under the measurement conditions, the test is not required. However, specify the reason.

3. ^{1}H-NMR and ^{13}C-NMR shall be measured concerning NMR.

Melting point test (2-9-5)

1. Conduct the test when observing solidification at about -20 degrees C. When the melting point is lower than 10 degrees C, the measurement method is not necessarily required to comply with the OECD test guideline.

2. If measurement is impossible due to, for example, thermal decomposition at temperature below the melting point during measurement, record the fact.

Boiling point test (2-9-6)

1. Conduct measurement as far as possible regardless of liquid/solid form.

2. When conducting thermal analysis, try to improve the measurement method by, for example, use of pinhole cells and/or correction based on the boiling point of the standard substance.

Vapour pressure test (2-9-7)

1. The test is not required for a substance whose boiling point is 30 degrees C or lower.

2. When conducting airflow analysis, take any actions to confirm saturation.

3. The test is not required when vapour pressure is estimated to be under 10-5Pa. However, specify the reason.

Test on solubility in water (2-9-8)

1. Set the maximum measured value at 250g/l and the minimum measured value at 10^{-6}g/l. When difficult to analyze, conduct measurement to the concentration as low as possible.

2. The test is not required when rapid decomposition is observed. However, specify the reason.

Test on solubility in organic solvent (2-9-9)

Set the maximum measured value at 250g/ l and the minimum measured value at 10^{-2}g/ l .

Soil adsorption test (2-9-10)

1. The preferable deviation rate in the equilibrium study shall be within 10% for each measurement period.

2. Obtain $K^{ads}_{F\ OC}$ dividing parameter K^{ads}_{F} of Freundliich's adsorption formula by organic carbon rate (OC%). ($K^{ads}_{F\ oc}=K^{ads}_{F}\div OC\%\times 100$)

n-octanol/water partition coefficient test (2-9-11)

1. When using a dissociable substance, conduct measurement under the non-dissociation conditions, for example, by using a buffer solution. Specify the reason and grounds.

2. When using the flask shaking method, conduct measurement under some conditions (e.g., phase ratio) to confirm being under the equilibrium condition.

Density test (2-9-12)

Conduct measurement of a true density, not a bulk density.

Hydrolysis test (2-9-13)

When decomposition is expected in the preliminary test, the main test may be conducted at first.

Dissociation constant test (2-9-14)

1. Dissociation shall be recognized as ionization in the solution including protonation.

2. Conduct the study within the practicable range such as alkaline pH.

3. The test is not required for the test substance whose solubility in water is no more than 10^{-4}g/l.

4. When using the titration method, describe the CO_2 removal method in the report.

5. The test is not required if the test substance is absolutely considered to be not dissociated at the normal pH range considering the chemical structure. However, specify the reason.

Thermal stability test (2-9-15)

1. Conduct measurement up to almost 400 degrees C.

2. When using the DSC or DTA method, attach a differential thermal chart.

3. An atmosphere gas for the DTA method is not particularly designated herein.

Test on photolysis in water (2-9-16)

1. When having a rational reason in conducting the test, the test using a buffer solution is applicable in place of distilled water.

2. Comply with Test on photolytic behavior in water.

Bioconcentration test (Fish bioconcentration test) (2-9-17)

1. Test organisms

(1) Organism species

① Common carp or Rice fish (*Oryzias latipes*) is recommended as test fish. However, other fish species is applicable when easily available, suitable sized and easy to breed in the laboratory.

② Use organisms whose history (e.g. the supply source and breeding method) is specified.

③ Preferably, use the test fish all of which are obtained from the same source, are at the same age, and have an uniform body weight as far as possible. Preferably, the lightest weight is at least 2/3 of the heaviest weight in the recommended total length of

test fish.
④ Select the number of test fish per test concentration such that a minimum of 4 fish per sample are available at each sample.
⑤ When using adult fish, report whether male or female, or both are used.

(2) Acclimatization
① Obtain the test fish by at least 14 days before the test and remove diseased and weaken fish.
② Conduct medicated bath treatment when obtaining fish if necessary. Refrain from any treatment including medicated bath during acclimatization or the experimental period.
③ Feed with the same diet as in the experimental period at least five times a week.

2. Exposure method

(1) In flow-through studies, employ the system that continuously supplies and dilutes a stock solution of the test substance in the test tank. Preferably, flush the test solution at least five times each test tank per day.

(2) Preferably, confirm the flow rate of the test stock solution and dilution water 48 hours before the test and every day throughout the experimental period.

3. Experimental period

(1) Experimental period should include the uptake phase and depuration phase (when calculating the concentration rate based on BCFk, etc.)

(2) Preferably, the uptake phase is 28 days unless the equilibrium obviously appears soon. If the steady-state is not observed during this period, extend the phase whichever early comes either until the steady-state is observed or up to 60 days for additional measurement.

(3) The depuration phase starts just after the uptake phase by transferring the test fish in the tank containing no test substance.
A half of the uptake phase is usually sufficient to eliminate at least 95% of the concentration of the uptake test substance in the steady-state.
If it takes excessive time to eliminate 95% (i.e., excess twice the uptake phase), it is allowable to shorten the period (until the day 90% is eliminated or at least twice the uptake phase).

(4) Even if calculating the concentration rate only based on BCFss, preferably set the depuration phase when BCFss is 1000 or more.

(5) The uptake phase and deputation phase may be estimated in advance based on Pow (partition coefficient (n-octanol/water)) or solubility in water of the test substance.
(See Reference 1)

4. Preparation of test solution

(1) When using a TGAI that is not water soluble, prepare the test stock solution by mechanical dispersion (e.g., ultrasonic treatment) or using commonly-used solubilizing agents such as N,N-dimethlyformamide, triethyleneglycol, acetone, ethanol, methanol and hardened castor oil. In this case, agents are not required to be completely dissolved but uniformly dispersed.

(2) Preferably, provide a solubilizing agent in the same concentration in all test tanks.

5. Environmental conditions

(1) Light

Light intensity or quality is not particularly specified herein. The light conditions usually employed in the laboratory are applicable.

(2) Feeding

Feed with the proper amount of diet every day during acclimatization and the experimental period to keep fish healthy and maintain body weight. The amount of the diet should be 1-2% of the body weight per day. Feed with a proper diet in which fat and total protein contents are specified.

Remove leftover and eliminated substances 30 minutes - one hour after feeding to keep the organic concentration in the tank as low as possible.

(3) Dilution water

To prevent the test substance from adhering on organic substances, use water which contains total organic carbon and natural particles as little as possible.

Specify the supply source and pretreatment method of the employed dilution water.

6. Observation and measurement

(1) Measurement of test substance concentration of fish

If it is difficult to respectively analyze each fish, pooling of the samples on each sampling occasion may be analyzed. In this case, preferably divide fish population into at least two groups to be analyzed.

(See Reference 2)

(2) Measurement of fat content of fish

When using adult fish of both sexes, preferably demonstrate that fat content does not differ between male and female fish or conduct measurement of fat content for each sex separately.

(3) Measurement of test substance concentration in test water

Collect water for analysis by suctioning through an inactive tube from the center of the tank.

(See Reference 2)

(4) Water quality

When using dechlorinated tap water or natural water as dilution water, preferably inspect dilution water quality prior to the test with reference to the water quality parameters (revised version: 2000), etc. Water quality may be periodically inspected.

Measure water temperature and dissolved oxygen concentration of the test solution at least once a week.

7. Result assay method

Calculate the concentration rate based on the measurement results of the concentration of the test substance contained in the test fish and the test water.

(1) BCFss

Plot the fish-containing concentration of the test substance during the uptake phase against time, and calculate BCFss using the following equation with fish-containing concentration (Cf) and concentration in water (Cw).

BCFss =Cf (average) in the steady-state / Cw (average) in the steady-state

(2) BCFk

BCFk is determined by the ratio of the coefficient of the uptake curve against that of the depuration curve. The depuration rate constant (k_2) is usually determined based on the depuration curve. Calculate the uptake rate constant (k_1) based on the fish-containing concentration (Cf) determined by the depuration rate constant (k_2) and the uptake curve.

BCFk =the uptake rate constant (k_1)/the depuration rate constant (k_2)

(See Reference 3)

8. Test report

(1) The items listed below shall be included for the test method.

① Exposure conditions

Test method (flow-through, semi-static); test nominal concentration and separation factor; preparation method of test solution (type and concentration of a solubilizing agent when using it); uptake and depuration phase

② Environmental conditions

Dilution water; test container and device; remained amount of test solution; water temperature; light; feeding information

③ Observation and measurement items

Observation items and method; measurement method of test substance concentration; measurement items and method of water quality; result assay method

9. Others

The items which would contact the test solution and/or dilution water should be made of the materials from which toxic substances are not eluded and on which the test substance is hard to adhere. Use a test container that is proper in volume and made of inactive materials such as glass. Use the same container in each experimental area.

(Reference 1)

○ Estimation of the uptake phase

Estimation of the elimination rate constant (k_2) and the required time for reaching the rate against the steady-state can be obtained prior to the test based on the experiential relationship between k_2 and the partition coefficient (n-octanol/water) (Pow) or the one between k_2 and solubility in water (s).

For example, k_2 (day^{-1}) can be estimated with the experiential formula (Note 1) given below.

$$\log_{10} k_2 = -0.414 \log_{10}(P_{ow}) + 1.47 \quad (r^2 = 0.95) \qquad \text{[Formula 1]}$$

Otherwise, use Kristensen Formula. (Note 2)

If the partition coefficient (P_{ow}) is unknown, estimation is possible with solubility of the test substance against water (s). (Note 3)

$$\log_{10}(P_{ow}) = -0.862 \log_{10}(s) + 0.710 \quad (r^2 = 0.994) \qquad \text{[Formula 2]}$$

Hereinbefore, s = solubility against water (moles/L): (n=36)

These formulas are applicable only to chemical substances whose Pow values is within 2-6.5. (Note 4)

The required time for reaching a certain rate against the steady-state may be obtained based on a general rate equation (first-order reaction kinetics) which describes intake and depuration with the estimated k_2.

$$\frac{dC_f}{dt} = k_1 \cdot C_w - k_2 \cdot C_f$$

If C_w is constant,

$$C_f = \frac{k_1}{k_2} \cdot C_w \left(1 - e^{-k_2 t}\right) \qquad \text{[Formula 3]}$$

When the steady-state is approaching ($t \rightarrow \infty$), Formula 3 can be approximated as given below. (Note 5, 6)

$$C_f = \frac{k_1}{k_2} \cdot C_w$$

That is,

$C_f / C_w = k_1/k_2 = BCF$

Then, $k_1/k_2 \cdot C_w$ is approaching to the fish-containing concentration ($C_{f,s}$) in the steady-state.

Formula 3 may be replaced as given below.

$$C_f = C_{f,\ s} \cdot \left(1 - e^{-k_2 t}\right)$$

That is,

$$\frac{C_f}{C_{f,\ s}} = 1 - e^{-k_2 t} \qquad \text{[Formula 4]}$$

Estimate k_2 by using Formula 1 or 2, and the required time to reach a certain rate against the steady-state can be estimated using Formula 4.

The statistically optimum uptake phase to obtain data (BCFk) which satisfies the statistical standard is the period to reach the intermediate point or $1.6/k_2$ or the period to reach 80% of the static state (limited to no more than $3.0/k_2$ or 90% of the steady-state) on the logarithmic curve plotting the fish-containing concentration of the test substance against time. (Note 7)

The time to reach 80% of the "steady-state" is calculated from Formula 4 as given below:

$$0.80 = 1 - e^{-k_2 t_{80}}$$

That is,

$$t_{80} = \frac{1.6}{k_2} \qquad \text{[Formula 5]}$$

The period to reach 95% of the "steady-state" is also obtained as given below.

$$t_{95} = \frac{3.0}{k_2} \qquad \text{[Formula 6]}$$

For example, the uptake phase up of the test substance with log P_{ow} = 4 is calculated from Formula 1, 5 and 6 as given below.

$\text{Log}_{10}k_2 = -0.414 \cdot (4) + 1.47$

$k_2 = 0.652$ days^{-1}

up (80pct)=1.6/0.652, that is, 2.45 days (59 hours)
or
up (95pct)=3.0/0.652, that is, 4.60 days (110 hours)

The uptake phase of the test substance with s=10^{-5}mol/L (log(s)=-5.0) is calculated from Formula 1, 2 and Formula 5, 6 as given below.

$\log_{10}(P_{ow}) = -0.862 \cdot (-5.0) + 0.710 = 5.02$
$\log_{10}k_2 = -0.414 \cdot (5.02) + 1.47$
$k_2 = 0.246$ days^{-1}

up (80pct)=1.6/0.246, that is, 6.5 days (156 hours)

or
up (95pct)=3.0/0.246, that is, 12.2 days (293 hours)
Otherwise, the time to reach the steady-state can be calculated from the following formula. (Note 7)

$$t_{eq}=6.54\times10^{-3}P_{ow}+55.31 \text{ (hours)}$$

○ Estimation of the depuration phase

The depuration phase should be the period to reach less than 5% of the steady-state. If it takes too long to practically reach less than 5% of the static state, the depuration phase shall be twice the usual uptake phase or more (i.e., 56 days or more), or a shorter period (e.g., the period for the test substance concentration to reach less than 10% of the steady-state). However, when using a substance whose intake-depuration pattern is more complicated than simple linear models, the longer elimination term may be employed to obtain the elimination rate constant. Note that the period depends on the time in which the fish-containing concentration of the test substance is the above the detection level of analysis.

The required period for the body-containing concentration to decrease to a certain rate of the initial concentration can be estimated from general equations (first-order reaction kinetics) which describe intake and depuration. (Note 2, 8)
Assuming C_w to be zero during the depuration phase, the equation may be simplified as given below.

$$\frac{dC_f}{dt}=-k\cdot c_f$$

That is,

$$C_f=C_{f,0}\cdot e^{k_2 t}$$

Where, $C_{f,0}$ is the concentration at the commencement of the depuration phase.
50% depuration will then be reached at the time (t_{50}) given by the following equation.

$$\frac{C_f}{C_{f,0}}=\frac{1}{2}=e^{-k_2 t_{50}}$$

That is,

$$t_{50}=\frac{0.693}{k_2}$$

95% depuration will be also reached at the time (t_{95}) as given below.

$$t_{95}=\frac{3.0}{k_2}$$

When setting 80% uptake ($1.6k_2$) during the uptake phase and 95% elimination ($3.0/k_2$) during the depuration phase, the depuration phase shall be as about twice as the uptake phase.

Note that calculations mentioned above are based on the assumption that the uptake-depuration pattern follows linear equations. When it does not follow a linear expression obviously, employ a further complicated model. (Note 1)

(Note 1) Spacie A. and Hamelink J.L: Alternative models for describing the bioconcentration of organics in fish. Environ. Toxicol. Chem, 1, 309-320 (1982)

(Note 2) Kristensen P.: Biofish-containing concentration comparison of BCF's derived OECD and ASTM testing methods; influence of particulate matter to the bioavailability of chemicals. Danish water Quality Institute (1991)

(Note 3) Chiou C.T. and Schmedding D.W.: Partitioning of organic compounds in octanol-water systems. Environ. Sci. Technol. 16(1) , 4-10 (1982)

(Note 4) Hawker D.W. and Connell D.W.: Influence of partition coefficient of lipophilic compounds on bioconcentration kinetics with fish. Wat. Res. 22 (6), 701-707(1988)

(Note 5) Branson D. R., Blau G. E., Alexander H. C. and Neely W. B.: Transactions of the American Fisheries Society, 104(4), 785-792 (1975)

(Note 6) Ernst W.: Accumulation in aquatic Organisms. In : Appraisal of tests to predict the environmental behavior of chemicals. Ed. by Sheehan P., Klein W. and Bourdeau P. H., 1985 SCOPE, John Wiley & Sons Ltd., New York, Part 4. 4pp 243-255

(Note 7) Reilly P. M., Bajramovic R., Blau G. E., Branson D. R., and Sauerhoff M. W., Guidelines for the optimal design of experiments to estimate parameters in first order kinetic models, Can. J. Chem Eng, 55, 614-622(1977)

(Note 8) Konemann H. and Van Leeuven K.: Toxicokinetics in Fish: Accumulation and Elimination of Six Chlorobenzenes by Guppies. Chemosphere, 9, 3-19(1980)

(Reference 2)

Exemplary sampling schedule for the fish bioconcentration test of substances with $logP_{ow}$ = 4.0

Fish Sampling	Sampling time schedule		Number of water samples	Number of sample fish for each time
	Minimal required for sampling (days)	Additional days for sampling (days)		
Uptake phase	-1 0		2* 2	 Add 45-80 fish
1st time	0.3	0.4	2 (2)	4 (4)
2nd time	0.6	0.9	2 (2)	4 (4)
3rd time	1.2	1.7	2 (2)	4 (4)
4th time	2.4	3.3	2 (2)	4 (4)
5th time	4.7		2	6
Depuration phase				Transfer fish into water not including the test substance
6th time	5.0	5.3		4 (4)
7th time	5.9	7.0		4 (4)
8th time	9. 3	11. 2		4 (4)
9th time	14. 0	17. 5		6 (4)

Remarks

- *: Conduct water sampling after flushing test water as at least three times as the tank volume.
- The bracketed values are the number of samples (of water or fish) when conducting additional sampling.

Note: When $logP_{ow}$ is 4.0, preparatory estimation of k_2 shall be 0.6521/day.
The total experimental period shall be 3 × uptake phase =3 × 4. 6 days, that is, 14 days.

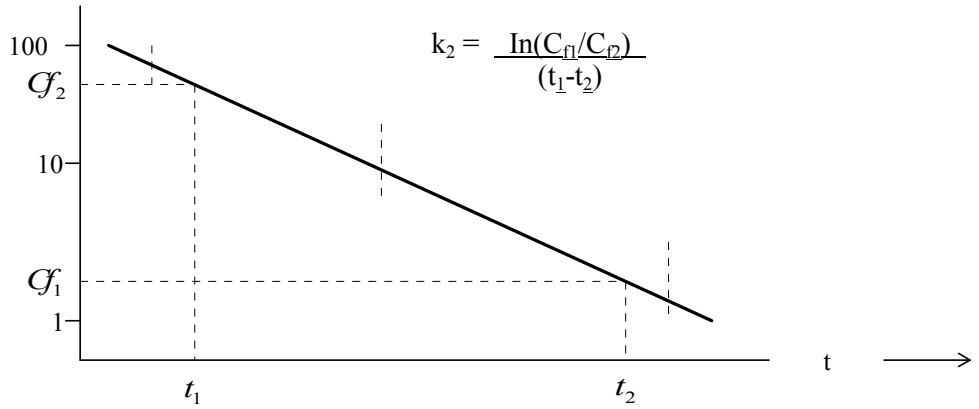
(Reference 3)

○ BCF calculation method

If an approximated curve plotting the fish-containing concentration during the depuration phase on the semilogarithmic paper becomes a line, it is considered to be practical to precisely describe the fish bioconcentration data on a simple model. (If these points are not described on a line, use a further complicated model.)

(1) Graphical method to determine depuration rate constant k_2

Plot the fish-containing concentration of the test substance at each sampling time on a semilogarithmic graph. The gradient of the line is k_2.



Note that a deviations from the line may represent the more complicated depuration pattern than a linear equation. The graphical method is useful to understand the form when the depuration does not follow the linear rate theory.

(2) Graphic method to determine uptake rate constant k_1.

Calculate k_1 from the following formula with k_2 as given above:

$$k_1 = \frac{C_f k_2}{C_w \times \left(1 - e^{-k_2 t}\right)} \qquad \text{[Formula 1]}$$

C_f is obtained from the center point of the uptake curve plotting the logarithmic concentration against time.

(3) Computer calculation method of intake and depuration rate constant

The more preferable method to obtain the fish bioconcentration and the rate constant of k_1 and k_2 is the computed nonlinear parameter estimation on a computer.

These programs calculate k_1 and k_2 in the following formulas from a pair of continuous time-concentration data:

$$C_f = C_w \cdot \frac{k_1}{k_2} \times \left(1 - e^{-k_2 t}\right) \qquad 0 < t < t_c \qquad \text{[Formula 2]}$$

$$C_f = C_w \cdot \frac{k_1}{k_2} \times \left(e^{-k_2 (t - t_c)} - e^{-k_2 t}\right) \qquad t > t_c \qquad \text{[Formula 3]}$$

Where, t_c = the ending time of the uptake phase

In this approach, the standard deviations of k_1 and k_2 are concurrently calculated.

In many cases, k_2 may be obtained from the depuration curve with relatively high accuracy. When concurrently calculating k_1 and k_2 between which a significant correlation exists, it is preferable to calculate only k_2 based on the depuration data at first, and subsequently calculate k_1 based on the uptake data using a nonlinear regression equation.

Test on derivation of predicted environmental concentration (2-10-1～6)

Test on water polluting properties (2-10-1)

1. On the Test paddies

(1) Preparation of test paddies

When preparing a new test paddies, note that the infilled soil layer shows neither difference between coarse and dense portions nor cracks. Filled with water after preparation, leave it until the soil becomes stable (for a couple of months).

(2) Select the test soil with as variable soil properties as possible.

Use the soil whose properties are specified such as: size distribution and soil classification (e.g., FAO/USDA); pH of soil (water and KCl solution or $CaCl_2$ solution); organic carbon content; CEC (cation exchange capacity); major clay mineral; other useful properties for evaluation of test results; and detailed information of sampling area (including history information). Findings of the soil groups (soil series groups) or component analysis would be one of useful information for evaluation of test results.

(3) Water used in test paddies

In case of river water, use one not including the active ingredient of the test substance. In case of tap water, use one which has remained untouched overnight. In these ways, don't use water containing substances which would have effects on analysis and decomposition of the active ingredient of the test substance.

2. On the Management of experimental area (test paddies)

When the test paddies lacks water, immediately refill.

If the test paddies are not provided with a roof, take care of flooding, etc. due to rainfalls.

3. On the Crop raised in experimental area

(1) The crop raised in the test paddies shall be the one among the crops that are to be used based on the description in the application form specified in the registration application of the agricultural chemicals. However, if the crop is too particular to be raised, the test may be conducted without raising the crop.

(2) The treatment term of the test substance shall be the term of use of the crops specified in the registration application.

(3) If there are plural crops specified in the registration application and employable methods, in principle, use the method which likely achieves the highest active ingredient concentration in water contained in the paddies.

4. On the Treatment and application of test substance

(1) For application of the test substance, use the method which may deal with the maximum treatment each time in active ingredient-equivalent amount.

When applying a diluting solution (e.g., emulsion), conduct calculation assuming that dispersion per 10a is 150 L.

When nursery boxes treatment, conduct calculation assuming 20 boxes are used per

10a.

(2) Preserve the test substance by proper means such as stoppers and seals, etc. Even if preserving for a long period after taking off a seal, it may not last more than one year.

(3) If the test substance cannot be applied immediately after preparation, apply it after a further preparation.

(4) Record weather conditions such as weather, rainfall, wind direction and wind speed when applying the test substance.

5. On the Sampling

(1) Conduct sampling using a random method based on a table of random numbers or systematic methods (e.g., S-shape or X-shape), except the edges of the experimental area.

(2) Confirm instruments to be clean prior to use for sampling.

(3) Start sampling and packaging from the non-treated area to prevent samples from being polluted by the hands, instruments or clothes supposed to contact the test substance.

(4) Package samples from each experimental area and take measurement to prevent from damages during transportation.

(5) Percolation water should also be sampled and analyzed during proper period (usually every one week) within the experimental term for water sampling contained in the paddies.

6. On the Treatment of samples

(1) When preserving samples, keep 5 ℃ or lower.

(2) When preserving samples, conduct the stock stability test.

(3) If floating substances (e.g., algae, daphnia, and humus) obviously exist in the test water, remove them by proper means such as filtration.

7. On the Analysis of samples

(1) Substances for analysis

① The substances to be analyzed shall contain the main metabolite formed during the test on behavior in soil and the test on behavior in water (usually referred to as the one whose formation rate is 10% or more, except for CO_2) in addition to the active ingredient of the agricultural chemicals. However, exclude the metabolite when it is known to have no toxic problems, is proven to have no toxic concerns by the toxicity studies, etc. (in principle, the acute toxicity and mutagenicity studies), or is considered to have no possibilities to remain.

② Preferably, the purity of the standard substance for analysis shall be approximately 95% or higher.

(2) Analysis method

The analysis method shall provide required accuracy, limit of quantification and recovery.

① Obtain the measured value by analyzing each sample twice or more and averaging the analyzed value.

② In principle, the standard deviation percentage (coefficient of variation = the standard

deviation ÷ average value × 100) shall have accuracy within 10% (however, 20% around the quantification limit) and the quantification limit no more than 1μg/L.

③ Quantification limit shall be the minimum concentration to obtain sufficient recovery when completing all operations for the sample. Adding the substance for analysis to the sample in the non-treated area to be as approximately the same to 1-10 times as the detectable limit, the concentration which obtains 70-120% recovery against the addition amount when completing all analysis operations shall be the quantification limit. Conduct analysis at least three times. Significant figures shall be within two digits.

④ Conduct measurement of recovery at the quantification limit concentration repeatedly at least three times by adding the active ingredient of the test substance to water contained in the paddies in the non-treated area. Conduct the recovery examination also at theoretical concentration obtained by the applied amount of the test substance and around the intermediate concentration between that and the quantification limit. In principle, describe the significant figures after rounded off to the whole number.

⑤ The detectable limit shall be, assuming that all of the operations for sample analysis was completed, the minimum concentration to clearly determine existence or nonexistence of the substance for analysis. The term "to clearly determine existence or nonexistence" means that, for example, the retention time of the substance demonstrates a distinct peak on a chromatogram which is not crossover with a sample-related interference peak, i.e., existence or nonexistence of the substance can be clearly determined in the analysis method. The significant figures shall be within two digits.

(3) Stock stability test

When preserving, in principle, recognize the decreasing amount of the stock substance for analysis to confirm not decreasing by concurrently preserving separately sampled water to which the known amount of the substance for analysis is added in the refrigerator. The recovery of the stock sample should be targeted at least 70% (without adjustment by the recovery test.)

8. On the Report

(1) Analyzed value

① Describe the analyzed value as it is without subtracting the value in the non-treated area nor adjusted based on the recovery.

② Round off the analyzed value to the digits in the quantification limit. The significant figures shall be within three digits.
Round off the figures according to the JIS Z8401-1999.

③ When the analyzed value is less than quantification limit (a μg/L), describe it as "<a μg/L".

④ When the analyzed value includes values less than quantification limit, don't average.

⑤ Convert the analyzed value of the metabolites to the active ingredient of the test substance.

⑥ The description method of the measured value shall comply with that of the analyzed value.

(2) Expected half life and calculation method

① Calculate the expected half life for the active ingredient of agricultural chemicals contained in the test substance. When metabolite may not be overlooked from the standpoint of its toxicity and residual amount, calculate the expected half life for the total amount of the measured value of all related metabolite converted to the active ingredient and the measured value of the active ingredient (when the measured value is the quantification limit or lower, add the quantification limit value).

② Calculate the expected half life by the least square method in principle, assuming that the active ingredient and metabolite decrease due to a primary reaction. Other methods are applicable if they properly calculate the expected half life.

(3) The report shall comply with "Analytical result report of water pollution" (Appended Format 1) and "Specification of analyzed sample preparation of water pollution" (Appended Format 2) and attach the attached sheet in the Appended Format 1.

Test on agricultural chemical concentration measurement in paddy water of model Paddy (2-10-2)

1. On the test paddy fields

This is the same as those of the Test on water polluting properties

2. On the control of the test plots (test paddy fields)

When the water in the test paddy field becomes insufficient, quickly replenish them with additional water. Attention must be paid to the water overflowing due to rain, when roofs are not used over the test paddy fields. It is preferable to keep the decrease of water per day by its penetration into the soil all during the test as little as possible.

3. On the crop plants that are cultivated in the test plots

(1) The crop plants cultivated in the test paddy field shall be chosen from those described in the application document when applying the agricultural chemicals for the registration. The test can be conducted without cultivating crop plants.

(2) The timing of the test substance preparation is same as that it is used for the crop plants that are applied for the registration.

(3) When a number of methods are used to apply for the registration, in principle use the method by which the concentration of the active ingredient, etc. in the paddy water is higher than those by other methods.

4. On the treatment and the use of the test substance

This is the same as those of the Test on water polluting properties.

5. On the collection of test samples (test paddy water)

(1) Collect samples so that the concentration in the paddy water during continuing five days can be grasped considering the duration of water outlet closing period.

(2) Select the places to collect test samples at random using a table of random numbers, or use systematic selection methods using S shape or X shape method etc. Do not collect test samples from the edge parts of the test plots.

(3) Confirm the cleanness of the apparatus, etc. used for test samples collection before using them.

(4) Start collecting and packing test samples from untreated plots and prevent the test samples from being polluted by collecting apparatus, human hands and cloths.

(5) The collected test samples shall be packed independently according to the test plots,

and careful attention must be paid so that the samples are not corrupted during transportation.

6. On the treatment of the test samples

This is the same as those of the Test on water polluting properties.

7. On the analyses of the test samples

(1) The substance to be analyzed

① The same substance at the one evaluated in toxicity tests concerning aquatic organisms in addition to the active ingredient of the agricultural chemical.

② Preferably, the purity of the standard substance for analysis shall be approximately 95% or higher.

(2) The method of analyses

This is the same as those of the Test on water polluting properties.

(3) Storage stability test

This is the same as that of the Test on water polluting properties.

8. On the report

(1) Values of analyses

Same as those of the Test on water polluting properties.

(2) Apply "Analytical result report of water pollution" and "Specification of analyzed sample preparation of water pollution" to this report, and attach "the attached sheet" to this report.

Test on agricultural chemical concentration measurement in paddy water of actual Paddy (2-10-3)

1. On the test paddy fields

(1) The selection of test paddy fields

① Two or more different paddy fields shall be used.

② As the paddy fields to be used for the test, select general paddy fields which have clarified soil properties.

③ Do not use inclined and significantly ill-shaped paddy fields. The replication of the test is not needed.

(2) The control of the test paddy fields

① The percolation runoff of water through levees shall be prevented as much as possible, and careful attention shall be paid to keep the decrease of water depth due to the levees percolation runoff to be equal to or less than 1 cm per day.

② Measure the decrease of the water depth per day appropriately and record it all during the test.

③ When the water in the test paddy fields becomes insufficient, quickly replenish them with additional water.

2. On the treatment and the use of the test substance

(1) The timing of the test substance preparation is same as that when it is used for the crop plants that are applied for the registration.

(2) When a number of methods are used to apply for the registration, in principle use the method by which the concentration of the active ingredients, etc. in the paddy water is the highest among those obtained by other methods.

(3) The test substance shall be tested using the method by which the amount of active ingredient by one preparation is maximized when shown in the converted value. In case of using diluted liquid like emulsifiable concentrate, etc., the amount shall be calculated on the condition that the application per 10a is 150 liter. When nursery boxes are used, the amount shall be calculated on the condition that 20 boxes are used per 10a.

(4) The test substance shall be kept properly in the containers that are sealed hermetically and tightly. It shall not be kept over one year even when it is stored for long after breaking of the seal.

(5) If the test substance cannot be used quickly after being prepared, it (the test substance) must be prepared anew before actually used.

(6) Record such weather conditions as weather, rainfall, wind direction, wind velocity etc. when the test substance is used.

3. On the collection of test samples

This is the same as those of the Test on water polluting properties in case of conducting tests to calculate Predicted Environmental Concentration for long-term risk assessment on human health. And, this is the same as those of the tests to agricultural chemical concentration measurement in paddy water of model paddy in case of conducting the test to calculate Predicted Environmental Concentration for short-term risk assessment on aquatic organisms.

4. On the treatment of the test samples

This is the same as those of the Test on water polluting properties.

5. On the analyses of the test samples

This is the same as those of the Test on water polluting properties in case of conducting tests to calculate Predicted Environmental Concentration for long-term risk assessment on human health. And, this is the same as those of the test to agricultural chemical concentration measurement in paddy water of model paddy in case of conducting the test to calculate the Predicted Environmental Concentration for short-term risk assessment on aquatic organisms.

6. On the report

(1) Values of analyses

Same as those of the Test on water polluting properties.

(2) Apply "Analytical result report of water pollution" and "Specification of analyzed sample preparation of water pollution" to this report, and attach "the attached sheet" to this report.

Test on surface soil runoff in model field (2-10-4)

1. On the test plots

(1) Test soil

The soil used for the test shall have clarified properties such as soil particle size composition, soil classification (FAO/USDA etc.), soil pH (water and KCl water solution or $CaCl_2$ solution), organic carbon content, CEC (cation exchange capacity), major clay minerals and other properties effective to assess the test results. The soil must also have clear information on the collected place and the history of the use concerning agricultural chemicals for the past three years. Pebbles and plant remainders must be removed from the soil.

(2) The water used for artificial rainfall

Do not use the water containing any substances that may influence the analyses and resolution, etc. of the active ingredients, etc. in the test substance.

2. On the control of the test plots

The test plots must be controlled so that such environmental conditions as the weather may be reflected to the test. Special attention shall be paid to the rainfall. The influence of natural rainfall must be avoided by moving the test field under roof when rainfall is forecast.

3. On the treatment and use of the test substance

(1) The test substance shall be tested using the method by which the amount of active ingredient in converted value is maximized at one preparation for the crop that is considered to cause the largest amount of surface runoff among all applied crops.

(2) When the application amount per unit area is not described on the instruction for application, investigate the actual cultivating situation of all crop plants and calculate the maximum application (use) amount.

(3) If the test substance cannot be used quickly after preparation, it (the test substance) must be prepared anew before actually used.

(4) Record such weather conditions as weather, rainfall, wind direction, wind velocity etc. when the test substance is used.

(5) The test substance shall be kept properly in the containers that are sealed hermetically and tightly. It shall not be kept over one year even when it is stored for long after breaking of the seal.

4. On the collection of test samples

(1) Confirm the cleanness of the apparatus, etc. used for test samples collection before using them.

(2) The collected test samples shall be packed independently according to the test plots, and careful attention must be paid so that the samples are not corrupted during transportation.

5. On the handling (treatment) of the test samples

(1) Keep the ambient temperature equal to or lower than 5℃ when storing the test samples.

(2) Storage stability test shall be conducted when storing test samples.

6. On the analyses of the test samples

(1) The substance to be analyzed

This is the same as those of the Test on water polluting properties in case of conducting tests to calculate Predicted Environmental Concentration for long-term risk assessment on human health. And, this is the same as those of the tests to agricultural chemical concentration measurement in paddy water of model paddy in case of conducting tests to calculate the Predicted Environmental Concentration for short-term risk assessment on aquatic organisms.

(2) The method of analyses

The method of analyses must have necessary accuracy, limit of quantification and recovery ratio.

① Analyze a test sample twice or more and make the average value of the analyses as the measured value.

② In principle, the coefficient of variation (standard deviation÷average value x 100) shall have the accuracy of within 10% (however 20% near the limit of quantification) and the limit of quantification shall be smaller than less than 1 μg/L.

③ The limit of quantification shall be the lowest concentration that gives sufficient recovery ratio when all operations are conducted to the test samples. Add the substance to be analyzed to the test samples in the non-treated plots so that they (the test samples) receive roughly 1 to 10 times of the detection limit. And make the limit of quantification as the concentration by which the recovery ratio to the total amount added during all the operation of analyses becomes 70 to 120 %. The analyses must be conducted three times or more. The significant figures shall be within two-digit numbers.

④ The recovery rate shall be measured three times or more continuously at the limit concentration of quantification after adding the active ingredients to the surface running water of the non-treated plots. The addition (of active ingredients) and recovery test shall be conducted at the theoretical concentration calculated by the used amount and the concentration near the middle between that and the limit of quantification. In principle, the significant numbers to show the recovery rate shall be integral numbers after rounding off the first decimal place.

⑤ The limit of detection shall be the minimum concentration by which the existence of the analyzed substance can be clearly acknowledged in case that all the operations of the analyses as to the test samples are assumed to be completed. The clear acknowledgement of the existence means that the existence of the object substance is clearly acknowledged by the applied analysis methods, for example, the maintaining time of the object substance shows clear peak on the chromatogram without overlapping the disturbance peak caused by the test samples. The significant figures shall be within two-digit numbers.

(3) Storage stability test

In case of storing test samples, the decrease of the analyzed test substance during the storage shall be measured while keeping the cold storage of other test samples which are made by adding known amount of analyzed substance to separately collected water. The target of the recovery ratio after the storage is equal to or larger than 70 %. (This shall not depend on the correction by recovery tests.)

7. On the report

(1) The values of analyses

Same as those of the Water Polluting Properties Test

(2) Runoff percentage

As to the result of analyses, calculate the runoff percentage at the timing of

collecting test samples by the average value of repeated analyses, and make a damping curve of the runoff percentage, and obtain the average runoff percentage using the damping curve. Add the runoff percentages of actually measured days and those obtained by the damping curve as to the days of no measurement, and then divide the total sum of the runoff percentages by the total number of test days. The result is the average runoff percentage.

Drift Test (2-10-5)

1. On the test fields

(1) In principle, use three or more different fields for the test.

(2) Provide sufficiently long interval between the tests when the same field is used for the test.

(3) There shall be no obstacles to cause any hindrance to the investigation in the investigation region of the test field.

2. On the crop plants cultivated in the test fields

(1) Select typical crop plants suitable for the purpose of assessment from those which are applied for the registration as the crop plants cultivated in the test field.

(2) In case that no field with cultivated crop plants is available, controlled fields of bare ground can be used.

3. On the treatment and use of the test substance

(1) Use formulations which are applied for the registration as the test substance. Select the spray apparatus, among typical spray apparatus for the object use, that is considered to provide the largest drift under the condition of dilution concentration that is the largest amount of sprayed active ingredients for the object use and the actually sprayed amount.

(2) In case that the test substance cannot be used quickly after being prepared, prepare it anew when it is actually used.

(3) Use the spraying apparatus according to its normal operation methods. Spraying must be done uniformly over all surface of the test field.

(4) At the spray, clarify the actual sprayed amount and do not fluctuate the spray over 10 % of the planned amount.

(5) The test substance shall be kept properly in the containers that are sealed hermetically and tightly. It shall not be kept over one year even when it is stored for long after breaking of the seal.

4. Weather observation etc.

(1) Provide anemoscopes and anemometers (at the height of 1.5 meter) at proper locations in the investigation plots, and measure the direction and speed of wind from the beginning of spray to the recovery of traps.

(2) As to the direction of wind, show the main wind direction and wind direction range as their angles to the traps arrangement direction. Concerning the wind speed, show the fastest, average and lowest speed during the spray.

(3) Record the weather, ambient temperature and humidity at the start of spray.

5. Measurement of droppage

(1) Locate five or more traps referring to the following.

For the agricultural chemicals used for paddy fields: 1*,3,5,6.5*,10,13*,16 and 20 meters from the boundary

For the agricultural chemicals used for the field other than paddy fields: 5,7,10,11.5*, 14, 18*, 23 and 30 meters from the boundary

Notes) It is preferable to locate traps on the locations marked with * above.

6. On the recovery of traps

(1) Avoid the pollution to the traps through apparatus, hands and cloths that might have contact with the test substance when recovering the traps.

(2) The recovered traps shall be united depending on the location (the distance from the boundary) and prevent them from any damages during transportation.

7. On the treatment of the test samples

(1) Keep the ambient temperature equal to or lower than 5℃ when the test samples are stored.

(2) Storage stability test shall be conducted when the test samples are stored.

8. On the analysis of the test samples

(1) The substance to be analyzed

The purity of the analyzed substance shall be considered to be higher than approximately 95%.

(2) The method of analysis

The method of analysis shall be provided with necessary accuracy, limit of quantification and recovery ratio.

① Add a fixed amount of proper solvent (something to assure high recovery rate) to the recovered traps and elute agricultural chemicals component inside the traps. Combine the eluted liquid from the traps that are located at the same distance and make the combined liquid as the test sample to be analyzed.

② The analysis shall be repeated twice or more for a sample and make the average as the measured value.

③ In principle, the coefficient of variation (standard deviation÷average value x100) has the accuracy of within 10% (however, within 20% near the limit of quantification).

④ The values of analyses shall be expressed in μ g/ m^2

⑤ The limit of quantification shall be the lowest concentration that gives sufficient recovery ratio when all operations are conducted to the test samples. The limit of quantification is the concentration by which the recovery ratio to the total added amount becomes 70 to 120 % when all the operation on the test samples are finished. The operation shall include the addition of 1 to 10 times of the analyzed substance in comparison with the limit of detection to the trap containers. The analyses must be conducted three times or more. The significant figures shall be within two-digit numbers.

⑥ The recovery ratio shall be measured repeatedly three times or more at the limit

concentration of quantification after adding active ingredients of the test substance into the trap containers. The addition and recovery test shall be conducted at the concentration of about 50 times of limit of quantification. In principle, the significant numbers to show the recovery ratio shall be integral numbers after rounding off the first decimal place.

⑦ The limit of detection shall be the minimum concentration by which the existence of the substance of analyses can be clearly acknowledged in case that all the operation of the analyses as to the test samples are assumed to be completed. The clear acknowledgement of the existence means that the object substance is clearly detected and acknowledged by the applied analysis methods for such example as that the maintaining time of the object substance shows clear peak on chromatographs without overlapping disturbance peak caused by the test samples. The significant figures shall be within two-digit numbers.

(3) Storage stability test

In case of storing test samples, the decrease of the analyzed test substance during the storage shall be measured while keeping the cold storage of other test samples which are made by adding known amount of analyzed substance to separate trap containers. The target of the recovery ratio after the storage is equal to or larger than 70 %. (This shall not depend on the correction by recovery tests.)

9. On the report

(1) The values of analyses

① Record values of analyses as they are. Do not correct them by using the recovery ratio.

② The values of analyses shall be described in the same digit as that of the limit of quantification. The significant figures shall be within three-digit numbers. The way of rounding off shall be according to JIS Z8401-1999.

③ When the values of analyses are smaller than the limit of quantification (a μ g/ m^2), describe them as [< a μ g/ m^2].

④ When the values of analyses include those smaller than the limit of quantification, do not use them for the calculation of the average values.

⑤ The describing method of measured values shall be same as that of the values of analyses.

(2) Drift ratio to the spray constituent (%)

Calculate the droppage amount per 1 square meters of the agricultural chemicals by the results of analyses, and indicate it as the drift ratio to the theoretical applied amount per square meter.

Monitoring test on agricultural chemical concentration in the rivers (2-10-6)

1. On the investigation districts

The penetration ratio shall be calculated as the ratio of assumed applied area calculated by the shipment of the agricultural chemicals and the application amount per unit area to the total cropping acreage.

2. On the collection of test samples (the river water)

(1) On the method of collecting

① Confirm the cleanness of the collecting apparatus, etc. before actually used.
② Collected test samples shall be packed on the basis of the collection sites and careful attention shall be paid not to corrupt them during transportation.
③ Collect the water from the same position of collecting places, and it is preferable to do it at the same time range every time.

(2) The period and interval of collecting test samples

① In case of using test samples for the assessment of water pollution

a. Agricultural chemicals used for paddy fields

When it is supposed that the agricultural chemicals are collectively applied at a district on a specific day or period, collect the samples on every several days during the period. The sample collecting at the dynamic observation points may end one month after the use of the agricultural chemicals is completed. If the measured values at the assessment points are sufficiently lower than the standard value (the assumed standard value if not stipulated) determined by the Minister of Environment while the chemicals are used, the sample collection can be finished one month after the period of using the chemicals.

b. Agricultural chemicals for the fields other than paddy fields

When it is supposed that the agricultural chemicals are collectively applied at a district on a specific day or period, collect the samples on every week during the period. If the measured values at the assessment points are sufficiently lower than the standard value (the assumed standard value if not stipulated) determined by the Minister of Environment while the chemicals are used, the sample collection can be finished one month after the period of using the chemicals.

② In case of using the test samples to assess the toxicity to aquatic organisms

a. The agricultural chemicals used for paddy fields

The sample collecting at the dynamic observation points may end one week after the use of the agricultural chemicals is completed. The sample collecting can be finished around one month after the period of using the chemical when the measurement at the assessment points is lower than or near the limit of quantification in the assumed period of high concentration.

b. The agricultural chemicals used for the fields other than paddy fields.

When it is supposed that the agricultural chemicals are collectively applied at a district on a specific day or period, collect the samples as frequently as possible during the period. Additionally, in case that the applied timing and number are extremely limited, the sample collecting can be finished around one month after the period of use of the agricultural chemicals.

3. On the measurement of flow rate and the observation of weather

As to the flow rate, when there are the observed data about the assessment points in advance or it (the flow rate) can be assumed by the data of the nearby places, those data can be used. If the data about the year when the test is conducted is not available, the data of past years can be used as alternative. Additionally, when the values at the assessed positions are sufficiently lower than the water pollution standard value (the assumed standard value if not stipulated) determined by the Minister of Environment in case of using the data for assessment of water pollution, the measurement shall be done once during the investigation. Weather, rainfall and ambient temperature shall be checked during the test period.

4. On the treatment of the test samples

This is the same as those of the Test on water polluting properties

5. On the analyses of the test samples

(1) The substance to be analyzed.

This is the same as those of the water polluting properties test in case of using the data for the assessment of the water pollution. And, this is the same as those of the test on the agricultural chemicals concentration measurement in paddy water of model paddy in case of using the data for the assessment of the toxicity to aquatic organisms.

(2) The method of analyses

The analyses shall have necessary accuracy, limit of quantification and recovery ratio.

① Analyze the same test sample repeatedly twice or more and make their average as the measured value.

② In principle, the coefficient of variation (standard deviation÷average value x100) shall have the accuracy of within 10% (however 20% near the limit of quantification), and the limit of quantification shall be one tenth of the acute effect concentration to aquatic organisms.

③ The limit of quantification shall be the lowest concentration that gives sufficient recovery ratio when all operations are conducted to the test samples. Add the substance to be analyzed to the test samples in the non-treated districts so that they (the test samples) receive roughly 1 to 10 times of the limit of detection. And the limit of quantification is the concentration by which the recovery ratio to the total amount added during all the operation of analyses becomes 70 to 120 %. The analyses must be conducted three times or more. The significant figures shall be within two-digit numbers.

④ The recovery ratio shall be measured three times or more repeatedly at the limit concentration of quantification after adding the active ingredients to the similar sample. The addition (of active ingredients) and recovery test shall be conducted at the concentration where the object agricultural chemicals are presumably detected. In principle, the significant numbers shall be integral numbers after rounding off the first decimal place.

⑤ The limit of detection shall be the minimum concentration by which the existence of the analyzed substance can be clearly acknowledged in case that all the operation of the analyses for the test samples are presumably completed. The clear acknowledgement means that the existence of the object substance is clearly acknowledged by the applied analysis methods for such example as that the maintaining time of the object substance shows clear peak on the chromatograph without overlapping disturbance peak due to the cause of the test samples. The significant numbers shall be two-digit numbers.

(3) Storage stability test

In case of storing test samples, the decrease of the analyzed test substance during the storage shall be measured while keeping the cold storage of other test samples which are made by adding known amount of analyzed substance to separately collected water. The target of the recovery ratio after the storage is equal to or larger than 70 %. (This shall not depend on the correction by recovery tests.)

6. On the report

(1) The ground on which the investigation districts are selected

It is expected to report the ground, on which the investigation districts are selected, such as the latest statistical data on the penetration ratio depending on the recent shipment (of the chemicals), utility situation and others. Also, the reason that the assessment points are chosen shall be reported.

(2) The situation of using the object agricultural chemicals in the investigation districts

The situation of using the object agricultural chemicals (constituents) shall be reported based on the control records or control guidelines etc. on the agricultural chemicals at the investigation districts and the use records of investigated agricultural chemicals

(constituents) obtained from five or more users at the district.

(3) The values of analyses
Same as those of the water polluting properties test

(4) Annual average concentration or the average concentration during the highest concentration period
① In case of using the average for the assessment of water pollution, the annual average concentration at the district of assessment shall be reported. Further, when the concentration of the test substance at the assessment points is sufficiently lower than the standard value of the water pollution determined by the Minister of Environment (assumed standard value if not stipulated) at the timing of use, it is not necessary to calculate the annual average.
② In case of using for the assessment of toxic affect to aquatic organisms, the report shall be as follows.
a. As to the chemicals used in paddy fields, report the maximum values of average concentration for continuous two days, three days and four days at the assessment points. (Even in the case of including no measurement days, the assumed values can be calculated if it is possible to assume the measured values using the actually measured values before and after the non measurement days.)
b. As to the chemicals used in the fields other than paddy fields, the maximum concentration at the assessment points during the investigation shall be reported. And, the average concentration similar to those of paddy fields shall be reported if the calculation is possible by using the investigation results. (in case that investigation was conducted frequently during the period of the maximum concentration.)

(5) Weather data during the period of the investigation
The situation shall be reported by using the data from nearby Amedas, etc. if the rainfall is considered to have influenced to the concentration of the river water.

<Persistence Test>
Test on residues in crops, etc. (3-1-1, 2)

Test on residues in crops (3-1-1)

1. On the test crop plants

As to the cultivating condition (glasshouse, open field, bagging and non-bagging), adopt glasshouse cultivation or non-bagging cultivation for the crop plants which are cultivated by these methods, or those for which it (glasshouse or non-bagging cultivation) will be supposedly used broadly in future.

2. On the test districts

(1) It is recommendable (or possible) for avoiding the contamination to provide buffer regions, barrier areas etc.

(2) It is acceptable to make a test field as different test districts by collecting test samples at different harvest times from the same test field.

3. On the cultivation of test crop plants

(1) In case that other agricultural chemicals must be applied for the disease and insects which infest during the test period, it is necessary to use the chemicals that do not hinder the analysis of the test agricultural chemicals.

(2) Do not use the crop plants as test samples when they do not grow to normal size because of drought etc.

4. On the treatment and use of the test samples

(1) The test substance shall be kept properly in the containers that are sealed hermetically and tightly. It shall not be kept over one year even when it is stored for long after breaking of the seal.

(2) If the test substance cannot be used quickly after preparation, it (the test substance) must be prepared anew before the actual use.

(3) Use general and conventional ways when spreader is used (for the application of the agricultural chemicals).

5. On the collection of test samples

(1) Select the places to collect test samples at random using a table of random numbers, or use systematic selection method using S shape or X shape method. Do not collect the test samples from the edge part of the test district. In case that the applied places for registration are paddy field levees, collect the test samples along the treated levees.

(2) Confirm the cleanness of the apparatus, bags etc. used for collecting test samples before actually using them.

(3) Start collecting and packing test samples from untreated districts and prevent the test samples from being contaminated by collecting apparatus, human hands, cloths etc.

(4) Careful attention shall be paid not to remove the test substance from the surface of test samples when handling them.

(5) The collected test samples shall be packed independently on the basis of the test districts (or each sample independently in the necessary cases), and careful attention must be paid not to corrupt them during transportation.

6. On the treatment of test samples

(1) Photographs must be taken immediately after receiving the test samples. The photographs must clearly show the size or the situation of the test samples.

(2) In case of storing the test samples, keep them in the temperature of 5 ºC or lower, and –20 ℃ or lower for cold storage and refrigerated storage respectively.

(3) Conduct the storage stability test when storing the test samples.

7. On the analyses of test samples

(1) The substances to be analyzed are the active ingredients of the object agricultural chemicals and major metabolites that are formed in the metabolism plant structure test etc. (Generally, those formed 10 % or more, but exclude CO_2) However, exclude the metabolites for which no concern about toxic problems is shown by the

result of toxicity tests (generally, acute toxicity or mutagenicity test) when there are no known toxic problem about them (the metabolites) among those metabolites. Also exclude them when it is judged that there is no residual decomposition products of the metabolites. The purity of analytical standard substance to be analyzed is approximately 95% or higher.

(2) The parts of test samples to be analyzed

① Mandarin oranges should be analyzed for the pulp and pericarp.

② As to crops listed in the left column of the following table, it is preferable to analyze portions described in the right column.

Crop name	portion for analysis
kiwi fruit	pulp and pericarp
pear, quince, and apple	fruit (excluding stylar scar, core and fruit peduncle) and stylar scar, core and fruit peduncle
loquat	pulp (excluding seed) and skin of fruit (excluding fruit peduncle)
peach	pulp (excluding seed) and skin
water melon, oriental melon and melon	pulp and skin of fruit (excluding vine)

note: to measure the weight of each portion.

③ As to stone fruits (e.g. apricots, Japanese apricots, Japanese plums, cherries, peach, nectarines, etc.), avocado, persimmon, mango, it is preferable to measure the weight of their seed.

④ As to tea leaves, it is also preferable to analyze extracts in compliance with the method of hot water extraction on the basis of food standard for reference.

⑤ As to Japanese radish for which the intended time of the agricultural chemical use is in the early stage of growth, the analyses shall be done for their leaves that are thinned or picked up. The test shall be conducted once or more for each.

(3) Analysis method

Other analysis methods can be used when it is presumably impossible to analyze properly by the methods stipulated at the establishment of the food standards. The methods of analysis shall have necessary accuracy, limit of quantitation and recovery ratio by method validation.

① The analyses shall be conducted twice or more for a test sample and make the average as the value of analysis.

② For recovery test two concentrations should be prepared by adding analyte to control sample. Recovery test should be repeated five times or more. The repeatability relative standard deviation (RSDr) and the mean of recovery ratio should be calculated. It is preferable to conduct recovery test using control samples from different locations.

③ The repeatability relative standard deviation (RSDr) and the mean of recovery ratio, depending on the concentration listed in the left column of table below, should meet standard in center column and right column in the same table.

Concentration (ppm)	Mean of recovery ratio (%)	RSDr
≦0.001	50-120	≦35
＞0.001～≦0.01	60-120	≦30
＞0.01～≦0.1	70-120	≦20
＞0.1～≦1.0	70-110	≦15
＞1.0	70-110	≦10

Repeatability relative standard deviation (RSDr) = standard deviation ÷ Average × 100

④ Limit of quantification should be around the range of 0.01-0.05 mg/kg (for forage crops to which a residual standard value for pasture grass applies, limit of quantification should be a concentration that would be in the range of 0.01-0.05 mg/kg if the moisture content of the crop is converted to 10%.). However, in the cases where a residual standard value for the agricultural chemical under the Specifications and Standards for Food, Food Additives, etc. (MHLW Notification No. 370 of December 28, 1959) has been established and where the agricultural chemical is difficult to analyze in the range of 0.01 ~ 0.05ppm, the limit of quantification should be set to one-tenth the standard value. The significant digits of the limit of quantification shall be less than two digits.

⑤ The detection limit shall be the lowest concentration by which the existence of the analyzed substance can be clearly acknowledged in case that all the operation of the analyses for the test samples are presumably completed. The clear acknowledgement means that the existence of the object substance is clearly acknowledged by the applied analysis methods for such example as that the retention time of the object substance shows clear peak on the chromatogram without overlapping the disturbance peak due to the cause of the test samples. The detection limit shall be calculated by using the sensitivity of the equipment to the test samples, collected amount of the test samples or concentration ratio by the analysis operation. The significant numbers shall be two-digit numbers.

⑥ In order to ensure the accuracy of test results, take necessary measures including accuracy control with reference to "General requirements for the competence of testing and calibration laboratories (ISO/IEC Guide 17025)" and "Analysis of Pesticide Residues: Guidelines on Good Laboratory Practice in Pesticide Residue Analysis (CAC/GL 40)" formulated by the Joint FAO/WHO Food Standards Programme (Codex Alimentarius Commission).

Following is examples of quality control.

<Note> Practical operation of the quality control on Crop Field Trial

【Internal quality control】

1. Every time of field samples analysis, one fortified control sample (level of fortification is 2-10 times of LOQ) and one control sample (untreated) should be analyzed. Recovery ration depending on the concentration listed in the left column of table below should meet standard in right column of the same table. In addition, In addition, to ensure that the analyte is not detected in untreated samples.

Concentration (ppm)	Mean of recovery ratio (%)
≦0.001	50-120
＞0.001～≦0.01	60-120
＞0.01～≦0.1	70-120
＞0.1～≦1.0	70-110
＞1.0	70-110

2. When sample analysis is carried out, the analysis should be repeated at least twice for the same sample. The repeatability relative standard deviation (RSDr) should be calculated from differences between results of analysis and confirm the result is within standard of RSDr or not.
   The criteria of repeatability relative standard deviation (RSDr) should be determined by each laboratory.

   For reference, an example of RSDr indication is showed as following

Example of determination of RSDr criteria
RSDr depending on the concentration listed in the left column of table below should lower than criteria in right column of the same table.

Concentration (ppm)	Repeatability relative standard deviation (RSDr)
≦0.001	35
＞0.001～≦0.01	30
＞0.01～≦0.1	20
＞0.1～≦1.0	15
＞1.0	10

In the case of analysis conducted by replicate, RSDr could be determined following numerical formula.
RSDr=difference between analytical value÷average × 100 × 0.89
See Also :「Bunseki gyoumuno kannritogijyutu」HIROSHI HAMAGUCHI (issued August 28, 1978)

However, even if RSDr exceeds the value of the right column of the table above, in the case of defference between analytical value is less than twice of limit of quantification RSDr is within a range considered.

3. Above 1. and 2. tests should be carried out in parallel by the same analyst who analyze field samples.

4. Outlines of recovery ratio and RSDr should be included in the study report.

5. Field samples should be analysed by person who performed the analytical methods validation.
In addition, it is desirable that field samples should be analysed without long interval after the analytical method validation.

【External Quality Assessment】
1. It is desirable to participate in the proficiency testing conducted by outside of the laboratory at least once every two years.
2. Summary of proficiency testing (time, provider, matrix, compounds to be quantified, result (such as Z scores), etc) should be stated in the report.

(References)
- General requirements for the competence of testing and calibration laboratories:ISO/IEC17025
- Guidelines on Good Laboratory Practice in Pesticide Residue Analysis:CAC/GL40

(4) On the storage stability test
① It is acceptable to conduct the storage stability test by using ground test samples after storing them (the analyzed samples) keeping their shape.
② The recovery ratio after the storage shall be 70 % or larger. (Do not use the corrected values after the recovery test.)

8. On the reporting items
(1) The result of analyses
① Describe the values of analysis in the non-treatment districts as they are and do not correct them using the recovery ratio.

② As to the analyzed values of metabolites, it is also needed to record the values converted to the parent compound based on the molecular weights of parent compound and metabolites.
③ The results of the analysis shall be arranged for each analyte and analyzed portion (whole conversion).
④ The values of analysis shall be shown in the same digit as that of the limit of determination, but the significant figure shall be equal to or within three. Rounding off the figures shall be as shown in the rule JIS Z8401-1999.
⑤ When the value of analysis is smaller than the limit of determination [a ppm], describe [< a ppm].
⑥ The detailed description on how the methods are modified shall be stated in case of using methods that are different from the notified methods of analyses developed at the establishment of the food standards.
⑦ The significant figure of the recovery ratio is in principle integral number after rounding off the first decimal place.
⑧ In the tests to judge if agricultural chemicals like antibiotic is detected, reports on the detection of such chemicals shall be attached as a reference even when the value of analysis is lower than the limit of determination.

(2) Copies of necessary certificates etc. as the inspection facilities shall be attached when following facilities are supposed to analyze the residual chemicals to the crop plants of small production.
① A facility that is registered as a registered inspection facility based on the stipulation of the 33rd clause of the Food Sanitation Law.
② A facility that is registered as a facility to measure and authenticate the concentration based on the stipulation of the 107th clause of the Measurement Law.
③ A facility that is approved as a certified facility to handle international test facility authorization standards.

9. On the Report

In principle, a report must contain the following items:

(1) Analysis information (①-⑨ should be prepared separately for each analytes.)
① Name of the laboratory which conducted analysis
② Name of test compound and formulation type
③ Name of Analyte
④ Name of test crop and portion of test crop to be analized
⑤ Summary of the analytical methods
⑥ Objectives of application
⑦ Concentration and amount used
⑧ Test field site
⑨ Analysis results (test samples, concentration and amount used, treatment date, sample collection date, sample receipt date, number of treatments, duration, number of analyses, sample analysis date, analytical values (maximum-minimum and mean) and duration between sample receipt and analysis)
⑩ Detail of analysis methods
a. Test compound and analytes (structural formulae, chemical names, and physical and chemical properties)
b. Analytical methods (reagents and instruments, sample preparation methods, operation conditions for analytical instruments, preparation of standard curves, analytical operations, limit of quantification and limit of detection, recovery, stability during storage, references, considerations (validation of analysis methods, flow charts of analysis, and sample weight

tables), attached reference tables and figures (such as chromatograms), and photos of crop plants of test samples

⑪ Summary of accuracy control

(2) Information on Field parts (sample preparation)

① Test compound
   a. Common name and formation type
   b. Names of and contents of the active ingredients
   c. Lot numbers of the test items

② Names of the test crop and variety

③ Name of the test facility and address of the test field

④ Soil texture (sand, sandy loam, loam, clay loam, or clay) and water requirement in depth (cm)

⑤ Record of the crops and applied agricultural chemicals in the field (in the most recent year)

⑥ Summary of the cultivation procedures
   Seeding time; transplanting time; type, amount, and time of fertilizer applications; age of tree; plant intensity (spacing between furrows or plants); number of plants (per 1000 m^2); water control; covering material (screen factor for tea plants); time of covering in strawberries or cherries, etc.; time of covering with cheese cloth or tunnels in tea plants; operational classification by outdoors/indoors and with/without bagging; and (suitable) harvesting period, etc.

⑦ Growth stage

⑧ Agricultural chemicals used other than the test item

⑨ Test areas
   a. Area and number of plants per test area
   b. Area, volume, and height in case of glasshouse cultivation
   c. Layout of test areas (showing the status of the entire test areas and neighboring fields, etc.)

⑩ Application methods
   For each test areas:
   Treatment date, concentration, and amount (per 1000 m^2 or per test group), growth stage at the time of treatment, treatment method, environmental conditions, etc. at the time of treatment (treatment time, general weather conditions on the treatment day including treatment time, effects of rain and wind on a spray test, instruments (machines) used for treatment, treatment to the trunk, water control in spray treatment in flooding, depth and soil water content in soil treatment, temperature and liquid ratio in seed disinfection, etc.)
   a. Use of spreader (treatment area, name and concentration or amount of spreading agents)
   b. Notes

⑪ Sampling
   For each treated plot:
   Sampling date, time, and weather at sampling, sampling order, sample amount for shipping, sample shipping date, general conditions of shipped samples (size (including variance), ripening stage (earlier, normal, or later), and other notable features and their causes)
   a. Sampling methods
      Describe the instruments (machines) used, detail of sampling methods, and transportation methods for dehydration after sampling, etc.
   b. Preparation and packing methods after sampling
   c. Sample transportation methods

⑫ Weather tables

(3) Appended format 4 must be attached to the report.

(4) Discussion on residues in crops (if necessary)

Test on persistence in soil (3-3-1, 2)

Test on residues in soil (3-3-1)

1. On the test field

(1) The test field shall have clarified information on the administration of agricultural chemicals before the test, that for the preceding crops, soil properties, etc. Two or more fields in this country with different soil properties shall be used for the test. If the applicants couldn't select the test fields with different soil properties on account of avoidable reason(s), they could select the test fields with different properties except for soil properties.

(2) The crop plants cultivated in the test fields shall be selected from the crops that are used for the application to register the agricultural chemicals.

(3) The test fields shall be selected with the consideration of the time of use, the method of use and the characteristics of chemicals when the crop plants are cultivated in the open field or some protected areas.

(4) When the crop plants that are applied for the registration vary to several different kinds such as vegetables and fruits, the crops that have more deposit to the soil shall be selected, and the test shall be conducted by cultivating the crop in the test field. Generally, vegetables are selected when there are both fruits and vegetables.

(5) When the applied places (fields) for registration are paddy field levees, non-cultivation paddy field or paddy field after rice harvesting, the test can be conducted with the condition of the upland fields.

(6) Do not use the agricultural chemicals that hinder the analysis of the component substance etc. of the object agricultural chemicals during the test period.

(7) Ordinal watering shall be continued after the harvest of the crop in case of using some protected fields.

2. On the use and treatment of the test substance

(1) When there are two or more methods of use relating to the registration application, it is possible to cancel the method which presumably has shorter half -life regarding the analyzed substance of the object chemicals. In that case, it is needed to show the ground to judge that the method has shorter or similar half-life in comparison with other methods when the agricultural chemicals are applied for the registration.

(2) The application amount per 10 are shall be 150 l for rice, 300 l for vegetables and 700 l for fruits when no concrete application amount is described for such

agricultural chemicals as emulsion type chemicals, etc. that are sprayed as they are after being diluted.

(3) As to the seed disinfection, the test shall be conducted by applying the amount of chemicals that is calculated using the amount applied to the test fields when the chemicals are used immediately after seeding or in the seeding boxes. Use 4 kg to seed unhulled rice and 20 raising seedling boxes.

(4) Adjusting the amount of application is acceptable to make analysis or calculation possible in following cases. (a) The applied amount of active ingredients of the agricultural chemicals calculated by the method that is applied for the registration is too little, and (b) it is difficult to analyze the concentration in the soil or to calculate the estimated half-life.

(5) When the registration is applied for use in the field before the paddy field plowing or it is applied for the use in the nontilled paddy field, the test shall be conducted by using suitable methods.

(6) The agricultural chemicals shall be used according to the contents described in the application documents at the registration of the chemicals, but other methods are acceptable when the methods shown in the contents are too special to use for conducting the test.

(7) When the test substance cannot be used quickly after the preparation, it (the test substance) shall be prepared anew before actually used.

(8) The test substance shall be kept properly in the containers that are sealed hermetically and tightly. It shall not be kept over one year even when it is kept for long after breaking of the seal.

(9) Record such weather conditions as weather, rainfall, wind direction, wind velocity etc. while the test substance is used.

3. On the collection of test samples

(1) The test period shall be in principle the duration in which the estimated half-life can be clarified. When the sample soil is collected for a year, it is presumable that the soil is plowed or spaded during that time, but in principle the sampling shall be continued without plowing during the period.

(2) The sample soil shall be collected from randomly selected places using random numbers table or the positions of collection shall be selected systematically using S-shape or X-shape method. Samples shall not be collected from the edge parts of the test plots.

(3) Confirm the cleanness of the apparatus, etc. to collect samples before starting to use them.

(4) Test sample collection and packing shall be started with the non-treatment plots, and prevent them (test samples) from being polluted through the hands, apparatus or cloths that may have contacted the test substance.

(5) The test sample shall not be dried by air-drying in principle. The sample soil must be crushed into small particles keeping field-moist soil situation. Exclude the gravel with particle diameter of 5 mm or larger and large organic substance from the soil

samples by using sieves. And then mix it well to make the sample for analysis. The soil of upland field shall be contained in polyethylene bags or glass bottles, etc. and packed. The soil of paddy field shall be contained in bottles etc. and packed.

(6) The collected samples shall be packed on the basis of test plots (in some cases, individually) independently, and careful attention shall be paid not to collapse them during transportation.

4. On the treatment of test samples

(1) The persons who prepare test samples shall write necessary items on the attached sheet 2 "Specification of Soil Residue Analytical Sample Preparation" of the Appended format 4 and send it to the persons to analyze them.

(2) The samples shall be analyzed as quickly as possible after collection. They can be stored in a freezer for unavoidable reason. Keep minus 20℃ or lower when storing them.

5. On the analysis

(1) The substance to be analyzed

The substance to be analyzed are the active ingredients of the object agricultural chemicals and major metabolites that are formed during the tests on the fate in soil, those in water etc. (Generally, the metabolites that are formed more than 10%, but carbon dioxide is not included.) But those metabolites shall be excluded when it is well known that they (the metabolites) have no toxicity, no toxicity is demonstrated as the result of toxicity test (generally acute toxicity test and mutagenicity test) and it is judged that there is no fear about residual decomposition of those metabolites. The purity target of the standard substance of analysis shall be approximately 95%.

(2) The method of analysis

The method of analysis shall have necessary purity, limit of quantification and recovery ratio.

① The analyses shall be conducted twice or more for a test sample and make the average as the value of analysis.

② As to the method of analysis, in principle the accuracy of standard deviation percent (variation coefficient = standard deviation ÷ average value x 100) shall be within 10 % (This value is 20 % near the limit of quantification) and the limit of quantification shall be equal to or smaller than 0.01 mg/kg. (This may be equal to or less than 1% of the concentration of the maximum value for active ingredient during the test period in unavoidable cases. As to metabolites, the figures shall not be converted to those of the parent compounds.)

③ The limit of quantification shall be the minimum concentration by which sufficient recovery ratio can be obtained when all the operations of the analysis are conducted. Add the substance to be analyzed to the test samples in the non-treated districts so that they (the test samples) receive roughly 1 to 10 times of the detection limit. And the limit of quantification shall also be the concentration by which the recovery ratio to the total amount added during all the operation of analyses becomes 70 to 120 %. The analyses must be conducted three times or more. The significant figure of the limit of quantification shall be within two (including two).

④ The recovery ratio shall be measured three times or more by using the concentration of the limit of quantification, the treatment concentration of the final test and the in- between of these two by adding the test substance to the soil of non-treatment districts. In principle, the significant numbers shall be integral numbers after rounding off the first decimal place.

⑤ The detection limit shall be the lowest concentration by which the existence of the analyzed substance can be clearly acknowledged in case that all the operations of the analyses for the test samples are presumably completed. The clear acknowledgement means that the existence of the object substance is clearly acknowledged by the applied analysis methods for such example as that the maintaining time of the object substance shows clear peak on the chromatogram without overlapping the disturbance peak due to the cause of the test samples. The detection limit shall be calculated by using the sensitivity of the equipment to the detection of test samples, collected amount of the test samples or concentration ratio by the analysis operation. The significant numbers shall be two-digit numbers.

(3) Storage stability test

In case of storing test samples, the decrease of the analyzed test substance during the storage shall be measured while keeping the cold storage of other test samples which are made by adding known amount of analyzed substance to separately collected soil. The target of the recovery ratio after the storage is equal to or larger than 70 %. (This shall not depend on the correction by recovery tests.)

6. On the report

(1) The values of analysis

① The values of analysis shall be described as they are without reducing those of the non-treatment plots. Correction using the recovery ratio shall not be added, either.

② The values of analysis shall have the same digit as that of the limit of quantification, but the significant figure shall be 3 digits. The figures shall be round off according to the rules of JIS Z8401-1999.

③ When the value of analysis is smaller than the limit of quantification [a mg/kg], describe [< a mg/kg].

④ The value of analysis smaller than the limit of quantification shall not be added to the calculation of the average.

⑤ The value of analysis shall be shown as the value per unit of oven-dry soil.

⑥ The value of analysis for metabolic decomposition product shall be converted to that of the active ingredient of the test substance, and both the values before and after conversion shall be described in the report.

⑦ The description method of the measured values is the same as that of the analyzed values.

(2) Estimated half life and its calculation

① Estimated half life shall be calculated for the active ingredients of the agricultural chemicals relating to the test substance. In case that there are metabolites that cannot be ignored as to the toxicity and residual amount, the half-lives shall be calculated concerning all the converted values of measurement and actually measured values of the active ingredients. (The limit of quantification shall be added when the measured value is smaller than the limit of quantification.)

② The estimated half-life shall be in principle calculated by using the least squares method on the assumption that the active ingredients and metabolites decrease by the first order reaction. Other methods can be adopted when it is possible to properly calculate half-life by using them.

(3) A report should be prepared according to "Analytical result report of residues in soil" (Attached Format 3) with references attached as appended sheet.

Test on residues in succeeding crops (3-3-2)

1. On the test crop plants

(1) Select the crop plants that have high possibility to be cultivated as the succeeding crop plants.

(2) Exclude perennial crops and the crop plants that are cultivated for long (longer than a year) in the same field.

(3) When there is concern about the chemicals residue in the succeeding crop or the test crop has higher residual chemicals than the limit of quantification, it is preferable to cultivate additional crop plants that have high possibility to be cultivated as succeeding crops. Select the crops for additional cultivation from the same kinds of crops which the (original) test crops belong to and root vegetables, leafy vegetables, fruiting vegetables, grains, pulse crops and tubers if they (selected additional crop plants) are not chosen as the original test crop.

2. On the test plots

(1) The methods and typical crops shall be selected for preceding cropping so that the residual amount in the soil is assumed to become the maximum among the crops that are scheduled to be applied for the registration at the start of cultivating the succeeding crop.

(2) If it is difficult to provide test fields for the preceding crops, it is acceptable to use the fields or cultivation pots when the total amount of active ingredients of the agricultural chemicals used in the method shown in above (1) is administrated into their soil. In this case, the time of planting the test crops shall be decided taking the period of administration to the preceding crops relating to the application for the registration into consideration. If the preceding crops are paddy field crop plants and it is assumed that the agricultural chemicals is used in flooded paddy field, the test field shall be kept as flooded for a certain period after adding the agricultural chemicals.

(3) In case of using cultivating pots, proper pots shall be selected so that proper cultivation of the test crop is possible.

3. On the analysis of the crop samples

(1) For the agricultural chemicals containing the substance of analysis that is stipulated in the food standard, the substance shall be the substance to be analyzed.

(2) What shall be added as the substance of analysis are the metabolites that cannot be ignored in terms of toxicity and residue amount among the substance of analysis shown in the residue in soil test.

(3) The target value of the limit of quantification is generally 0.01 ppm. The standard value shall be set up for the agricultural chemicals that have the standard value smaller than 0.01 ppm.

(Appendix Table1-1)

Applicable crops (Crops that are to be used for food (including industrial crops and crops to be used for animal feed): Crops for which test results regarding residue in crops are required.)

Large crop group name	Medium crop group name	Crop name	Examples of alias name, local name, variety name, etc. included in the crop name	Remarks
Rice		Rice	Paddy rice (transplanting, direct sowing), upland rice	Crops whose seeds are harvested
Cereal grains		Oats	*Otomugi, enbaku, karasumugi*	
		Barley	*Nijhoshu, rokujhoshu,* naked barley	
		Wheat		
		Rye		
Cereals(others)	Small-grain cereals of the Poaceae	Millet, Foxtail		
		Millet,Common		
		Millet, Barnyard		
	Corn	Maize		
		Immature corn	Sweet corn	Crops whose seeds (partly mature ear) are harvested
		Amaranth or Pigweed	*Himogeito, senninkoku, sugimorikeito, fujigeito, hansuihiyu, shuryuhiyu*	Crops whose seeds are harvested
		Quinoa		
		Edible sorghum	*Morokoshi, takakibi, koryan*	
		Buckwheat	*Dattansoba*	
		Job's tears		
Fruit trees	Citrus fruits	*Amakusa*		Crops whose fruits are harvested
		Encore		
		Iyokan		
		Obenimikan		
		Orange	Sweet orange, Valencia orange	

		Karbuchie		
		Kabosu		
		Kara	*Kara* mandarin	
		Kawachibankan		
		Kiyomi		
		Kumquat	*Ninpokinkan*, *marukinkan*	
		Grapefruit		
		Saga mandarin		
		Summer fresh		
		Shekwasha		
		Jabara		
		Shiranui	*Dekopon*	
		Sudachi		
		Setoka		
		Seminor		
		Tarogayo		
		Tankan		
		Nagatoyuzukichi		
		Chinese citron	Amanatsu, natsudaidai	
		Navel orange	Washington navel orange	
		Hassaku orange		
		Haruka		
		Harumi		
		Harehime		
		Hyuganatsu		

		Shaddock	*Zabon, bontan, banpeiyu, uchimurasaki*	
		Hebesu		
		Ponkan mandarin		
		Marcot		
		Mandarin orange	Satsuma mandarin(*Citrus unshiu*)	
		Yuzu		
		Lime		
		Lemon		
	Small stone fruits	*Anzu* (*Prunus armeniaca*)	Apricot	Crops whose fruits are harvested
		Japanese apricot (*Prunus mume*)		
		Japanese plum (*Prunus salicina*)	Plum, prune	
	berries	Aronia	Choco berry	Crops whose fruits are harvested
		Edible mulberry (fruit)	*Karaguwa, yamaguwa*	
		Seaberry	Saji, sarji, sunajigumi	
		Goosebelly		
		Blue honeysuckle	*Kurominouguisukagura*	
		Fusasuguri	*Currant, karanto, karantsu akafusasuguri, kurofusasuguri, cassis*	
		Blackberry		
		Blueberry		
		Raspberry		
		Akebi (fruit)		Crops whose fruits are harvested
		Acerola		
		Atemoya		
		Avocado		
		Fig		

		Gingko (seed)	*Ginnan*	Crops whose seeds are harvested
		Cherry	*Sakuranbo*	Crops whose fruits are harvested
		Olive		
		Japanese persimmon		
		Canistel	Egg fruit, *kudamonotamago*	
		Gamazumi(*Viburnum dilatatum*)	*Jomi*	
		Chinese quince		
		Kiwifruit		
		Guava (fruit)	*Banjiro, banzakuro*	
		Dwarf Japanese quince	*Sudome, shidomi*	
		Japanese chestnut		Crops whose seeds are harvested
		Walnut		
		Gorenshi (*Averrhoa carambola*)	Star fruit	Crops whose fruits are harvested
		Pomegranate		
		Sapodilla		
		Sarunashi (*Actinidia arguta*)	*Kokuwa, kosui*	
		Hawthorn (fruit)	*Oosanzashi, oomisanzashi*	
		Japanese pepper (fruit)		
		Jabuticaba		
		Edible camellia (seed)		Crops whose seeds are harvested
		Strawberry guava		Crops whose fruits are harvested
		Cherimoya		Crops whose fruits are harvested
		Pear	Japanese pear, European pear, Chinese pear	
		Jujube		
		Nectarine	*Aburamomo, zubaimomo*	
		Pineapple		

		Passion fruit	*Kudamonotokeiso*	
		Banana		
		Rugosa rose (fruit)		
		Papaya		
		Sugar apple	*Shakato, atesu,* suger apple	
		Pitaya	White pitaya, red pitaya, golden pitaya, dragon fruit	
		Surinam cherry	*Tachibanaadeku,* surinamu cherry, brazilian cherry	
		Loquat		
		Feijoa	Pineapple guava	
		Grape	Small fruit type grape (Delaware, *shiragabudo*, *yamabudo*), large fruit type grape (*Kyoho* type tetraploid varieties, diploid American varieties, diploid European varieties, triploid varieties, etc.) (Note 1)	
		Pecan		Crops whose seeds are harvested
		Pepino		Crops whose fruits are harvested
		Pawpaw	*Akebigaki*	
		White sapote		
		Quince		
		Mango		
		Miracle fruit		
		Japanese staunton-vine		
		Peach		
		Bayberry		

		Ryugan	Longan	
		Apple		
		Lychee	*Raichi*	
		Wax jambu apple	*Jawa-futomomo*	
Vegetables	Cucurbitaceous fruit (to be used for pickles)	*Akageuri*	*Mowi*	Crops whose fruits are harvested
		Edible bottle gourd		
		Edible loofah		
		Oriental pickling melon(*Cucumis melo* ver. *Conomon*)	*Aouri, karimori, hagurauri, aoshimauri*, kurouri, *katsurauri*	
		Watermelon to be used for pickles	*Gengobe-suika*	Crops whose immature fruits are harvested
		Oriental melon be used for pickles	*Becchin uri*	
		Melon to be used for pickles		
		Wax gourd	*Kamouri, togan*	Crops whose fruits are harvested
		Chayote		
		White flowered gourd	*Kanpyo*	
	Chili peppers	*Amanagatogarashi*	*Fushimi-togarashi, manganji-togarashi, Sanpo-togarashi*	Crops whose fruits are harvested. Sweet type to be used while they are immature.
		Kagurananban		Crops whose fruits are harvested Pungent type to be used while they are immature or ripe.
		Kidachitogarashi		
		Shishito pepper	*Shishitogarashi, shishito, aoishishito*	
		Red pepper	*Takanotsume, hachifusa, nikko togarashi, sapporo-onagatogarashi*	
		Habanero pepper		Crops whose fruits are harvested
		Picante		
	Nabanas	*Asama-kona*	*Asakumakona*	Crops whose stems and leaves, and flower buds are harvested
		Asukko		
		Aletta		
		Osakina	*Osakina*	
		Otakabu	*Yaruna*	

		Autumn poem	*Asuparana*	
		Onona		
		Kairan		
		Kakina	*Kakina, miyauchina, miyazakina , cona*	
		Katsuyamamizuna	*Heisenji-mizuna, gunsuina, sanmaimizuna, Kitaichimizuna*	
		Kukitachina	*Aizu-kukitachina, kaburena, chirimen-kukitachina*	
		Kosaitai	*Kosaitai*	
		Asiasarum root	*Saishin, aburasaishin*	
		Sankeiyukina		
		Shisen-asai	*Komochitakana*	
		Bok choy (nabana-like cultivtation)		
		Tsuminabana		
		Tsubomina	*Tsubomina, sanriku tsubomina, adeyaka tsubomina*	
		Nabana	*Nanohana, hanana,*	
		Norabona	*Norabona*	
		Hakatatsubomina		
		Hatakena (nabana- like cultivation)	*Hatakena* (nabana-like cultivation)	
		Hanakkori		
		Mizukakena	*Mizukakena*	
		Meikena	*Meikena*, niigatanabana	
	Non-head brassica leafy vegetables	*Azamina*	*Chirimen-karashi, Hagoromo-karashina*	Crops whose stems and leaves (before their scapes start to grow) are harvested
		Ajimina		
		Umino (stem and leaves)		
		Oyamasodachi		
		Black cabbage	*Kurokyabetsu*	
		Kahokuna	*Kahokuna*	
		Indian mustard	*Ki-karashina, ha-karashina, Yamashiona,* Red asian mustard, Green mustard, serifon	
		Kisona		
		Kale	*Hagoromokanran, Ryokuyokanran*	
		Komatsuna(*brassica rapa* var. *Perviridis*)	*Komatsuna*	

	Sagami green		
	Sanukina		
	Santosai	Santosai, bekana, hikekkyu-hakusai, Hankekkyu-hakusai, igamurasaki	
	Shisen-zasai (stem and leaves)	*Takenokotakana*	
	Shirona	*Osaka-shirona, naniwana, kyonosato-shirona, inamina*	
	Sendai bashona		
	Sendai yukina		
	Senposai 1-go	*Senposai 1-go*	
	Senposai 2-go	*Senposai 2-go*	
	Tarsai	*Tarsai, kisaragina, hisagona, chijimina*	
	Taisai	*Taisai, taina,seppakutaisai, nikanmetaisai, shakushina, nagaokana*	
	Leaf mustard	*Takana, katsuona, seisai, yamagata-aona, miike-takana*	
	Tabetena		
	Bok choy	*Chingensai*	
	Tegorona		
	Nagasaki-hakusai,	*Nagasaki-hakusai, tojinna, karana*	
	Nakajimana	*Nakajimana*	
	Nozawana	*Nozawana*	
	Hakatahakusai		
	Pak choi		
	Hatakena	*Hatakena*	
	Hikoshima –haruna	*Hikoshimaharuna*	
	Vitamin-na		
	Himejiwakana		
	Hiroshimana	*Hiroshimana*	
	Benrina		
	Mizuna (*brassica rapa* var. *Nipposinica*)	*Kyona, mizuna, kyomizuna*	
	Mibuna	*Mibuna*	
	Yamagata-midorina		

	Yamatona	*Yamatomana*	
	Arugula	Rocket, *selvachiko*, *kalgieru,* eruca	
	Wasabina		
Non-head lettuces	*Kakichisha*	Sanchu, chimasanchu	Crops whose leaves are harvested
	Lettuce	Butter-head-type-lettuce, butter-head-type-*tamajisha*	Crops whose stems and leaves are harvested
	Tachichisha	*Lomein* lettuce, cos lettuce	
	Bimitasu	*Bimitasu*	
	Leaf lettuce	*Hachisha, chirinenchisha,* sunny lettuce, silk lettuce, frill lettuce	
Legume vegetables	*Edamame*(immature soybean in the pod)		Crops whose immature peas with pod are harvested
	Common bean(pods and immature seed)	*Hirazaya-ingen Morocco-ingen*	
	Podded pea	*Kinusaya-endo, sunakku-endo, sato-endo, snap-endo*	
	Peas(succulent seed)	*Usui-endo*, green pea	Crops whose immature seeds (including those harvested with pod) are harvested
	Immature cowpea	*Juroku sasage, akishima-sasage*	Crops whose immature peas with pod are harvested
	Immaturewinged bean	*Urizun, tosai*	
	Immature broad bean		Crops whose immature seeds (including those harvested with pod) are harvested
	Immature sword bean		Crops whose immature peas with pod are harvested
	Immature hyacinth bean	Immature sengokumame, immature kaga-tsurumame, immature tsurumame	
Leafy vegetables of the Lamiaceae	*Egoma* (*perilla frutescens* var. *Frutescens*) (leaf)		Crops whose stems and leaves are harvested
	Oregano	*Hanahakka*, wild majoram	

		Shiso(*perilla frutescens* var. *Crispa*)	*Ooba*	
		Sage(*salvia officinalis*)	Common sage , Pineapple sage, Cherry sage , Red sage	
		Thyme	Common thyme, creeping thyme, lemon thyme, (persian hyssop), orange time, （sauce time）	
		Basil	Sweet basil, dark oparl basil, basil, *meboki*	
		Mint	Mint, spearmint, peppermint, apple mint	
		Lemon balm	Seiyo-yamahakka	
		Rosemary	*Mannenro*	
	Leafy vegetables of the Apiaceae	*Kinsai*	Soupcelery ,*shansai*, *chugoku-zeri, kintusai*	Crops whose stems and leaves are harvested
		Coriander (leaf)	*Kosai, shantuai, pakuchi, koendoro*	
		Japanese parsley		
		Celery		
		Chervil	*Uikyo-zeri serfeiyu*	
		Dill (leaf)	*Inondo, jira*	
		Carrot (leaf)	*Haninjin*	Crops whose comparatively young leaves are harvested with roots
		Parsley	Moss curled parsley, italian parsley	Crops whose stems and leaves are harvested
		Fennel (leaf)	*Uikyo*	
		Mitsuba(japanese honewort)	*Ao-mitsuba, kiri-mitsuba, Ne-mitsuba*	
		Artichoke	*Chosen-azami*	Crops whose flower buds are harvested
		Iceplant		Crops whose stems and leaves are harvested
		Akebi (stem and leaf)	*Kinome*	Crops whose stems and leaves (young buds) are harvested

		Asatsuki (*Allium schoenoprasum* var. *foliosum*)	*Itonegi, Senbonwakegi*	Crops whose leaves and bulbs are harvested
		Ashitaba.		Crops whose stems and leaves are harvested
		Asparagus		Crops whose shoots are harvested
		Sweet hydrangea leaf		Crops whose stems and leaves are harvested
		Amaranth (leaf)	Hageito, aobiyu, honagainubiyu, hiyuna	
		Strawberry	*Oranda-ichigo*	Crops whose fruits are harvested
		Gingko (leaf)		Crops whose leaves are harvested
		Ukogi	*Ukogi (yamaukogi) , himeukogi*	Crops whose stems and leaves are harvested
		Turmeric	*Aki-ukon, kyoo, haru-ukon*	Crops whose rhizomes are harvested
		Seibold's wild ginger		Crops whose rhizomes and roots are harvested
		Udo		Crops whose young parts of stems and leaves are harvested
		Oriental senna (stems and leaves)	*Rokkakuso*	Crops whose whole grasses above ground are harvested
		Ensai	*Entsai, asagaona, kushinna, tsuuna, yosai*	Crops whose stems and leaves are harvested
		Endive	*Nigachisha, kikuchisha*	
		Ogi	*Kibanaogi, Naimoogi*	Crops whose roots are harvested
		Goldthread		Crops whose rhizomes are harvested
		Okanori		Crops whose stems and leaves are harvested
		Saltwort	*Kusahijiki, okamiru, miruna*	
		Okra		Crops whose fruits are harvested
		Okera	*Oobanaokera, hosobaokera*	Crops whose rhizomes are harvested
		Oranda-waremoko	Salad barnet	Crops whose leaves are harvested
		Olive (leaf)		
		Kaensai	Detroit dark red, red beet, garden beet	Crops whose roots are harvested
		Japanese persimmon (leaf)		Crops whose leaves are harvested
		Valerian	*Haru-ominaeshi*	Crops whose

			rhizomes and roots are harvested
	Turnip	*Akakabu, akana, atamikabu, inekokina, ookabu, onikubina, gensuke-kabuna, kokabu, Oushida-na, Shogoin-kabu, (sugukina), Chozenji-na, Tsuda-kabu Tennoji-kabu, Narusawa-na, habirona, Hinona-kabu(hinona), Fukushima-na, benikabu, yurugikabu*	Crops whose stems and leaves, and roots are harvested
	Squash	*Nihon-kabocha, seiyo-kabocha, pebo-kabocha,(except zukkini),*	Crops whose fruits are harvested
	Chamomile	*Kamomairu, kamitsure,* german chamomile, roman chamomile	Crops whose flowers are harvested
	Cauliflower	*Hanayasai*, *sangosho*, romanesco	Crops whose flower buds and scapes are harvested
	Curry plant		Crops whose stems and leaves are harvested
	Kawaraketsumei		Crops whose whole grasses above ground (stems and leaves, and legumes) are harvested
	Sugar cane (stem and leave)		Crops whose stems and leaves are harvested
	Daylily (flower)	*Shinakanzo, nokanzo, yabukanzo, kibanana, kinshinsai*	Crops whose buds are harvested
	Chrysanthemum (leaf)	*Kikuha*	Crops whose leaves are harvested
	Plantain lily	*Urui*	Crops whose stems and leaves are harvested
	Cabbage	*Chirimen kyabetsu, saboi kyabetsu*	Crops whose stems and leaves after headed up are harvested
	Caraway (leaf)	*Himeuikyo*	Crops whose stems and leaves are harvested
	Cucumber		Crops whose fruits are harvested
	Cucumber (flower)	*Hanamarukyuri*	Crops whose female flowers are

			harvested
	Cucumber (leaf))		Crops whose leaves are harvested
	Longrooted onion		Crops whose leaves and bulbs are harvested
	Guava (leaf)		Crops whose leaves are harvested
	Kukichisha	*Yamakurage, tomusha*, stem lettus	Crops whose stems and young leaves in upper parts are harvested together
	Stem broccoli	Stick senor	Crops whose flower buds and scapes are harvested
	Chinese wolfberry (fruit)	*Kukoshi*	Crops whose fruits are harvested
	Chinese wolfberry (leaf)	*Kukoyoshi*	Crops whose leaves are harvested
	Ostrich fern	*Kogomi, gansoku, kogome, kugumi, niwasotetsu*	
	Cresson	Watercress	Crops whose stems and leaves are harvested
	Kuwai (*Sagittaria trifolia* var. *Edulis*)		Crops whose tubers are harvested
	Cranesbill		Crops whose whole grasses are harvested
	Konitabirako		Crops whose stems and leaves are harvested (called "*hotokoenoza*" in the seven spring plants
	Kohlrabi	*Kyukei-kanran, hakyabetsu*	Crops whose enlarged stems are harvested
	Koganebana		Crops whose roots are harvested
	Burdock		
	Comfrey	*Symphytum*, hirehariso	Crops whose leaves are harvested
	Zasai	*Kukitakana*, *umino* (enlarged stems), *shisen-zasai* (enlarged stems)	Crops whose enlarged stems are harvested
	Taro (*Colocasia esculenta*) (leaf stalks)	*Zuiki, datsuimo*	Crops whose leaf stalks are harvested
	Saffron		Crops whose pistils are harvested
	Sawa-azami	*Maazami*	Crops whose leaves are harvested
	Japan pepper tree (leaf)	*Kinome*	
	Chinese foxglove		Crops whose rhizomes are harvested
	Shiso (spike)		Crops whose spikes are harvested
	Chinese peony (for		Crops whose roots are harvested

		medical use)		
		Garland chrysanthemum	*Kikuha*	Crops whose stems and leaves are harvested
		Water shield		
		Ginger	*Neshoga*	Crops whose rhizomes are harvested
		Edible adiantum	*Ezogiku*	Crops whose leaves are harvested
		Edible aster		Crops whose flowers are harvested
		Edible scentedsolomom's seal		Crops whose rhizomes are harvested
		Edible aloe	*Curaso* aloe, cape aloe	Crops whose leaves are harvested
		Edible busy Lizzie	*Africa hosenka*, New Guinea impatience	Crops whose flowers are harvested
		Edible carnation		
		Edible maple leaf		Crops whose leaves are harvested
		Edible chrysanthemum		Crops whose flowers are harvested
		Edible snapdragon	Snapdragon	
		Edible calendula	Calendula	
		Edible mulberry (leaf)		Crops whose leaves are harvested
		Edible laurel	Laurier	
		Edible cosmos		Crops whose flowers are harvested
		Edible Japanese cherry (leaf)	*Sakuraba*	Crops whose leaves are harvested
		Edible cineraria	*Fuukigiku, cineria*	Crops whose flowers are harvested
		Edible sour dock	*Soreru, ozeiyu*	Crops whose leaves are harvested
		Edible garden stock		Crops whose flowers are harvested
		Edible puslane		Crops whose stems and leaves are harvested
		Edible dandelion	Edible *tanpopo*	Crops whose leaves are harvested
		Edible globe amaranth	*Sennichiko*	Crops whose flowers are harvested
		Edible torenia		
		Edible nasturtium	*Nozenharen Kinrenka*	
		Edible pink		

		Edible pansy		
		Edible primrose	Edible *sakuraso*	
		Edible petunia		
		Edible safflower (flower)		
		Edible winter-cherry	Golden berry, *tomatillo*, grand cherry	Crops whose fruits are harvested
		Edible miniature rose		Crops whose flowers are harvested
		Edible cornflower		Crops whose flowers are harvested (Species different from wild grass "*yagurumaso*")
		Edible lily	*Yurine*	Crops whose bulbs are harvested
		Edible lavender	English lavender	Crops whose stems and leaves, and flowers are harvested
		Edible rudbeckia	*Ohangonso*	Crops whose flowers are harvested
		Watermelon		Crops whose mature fruits are harvested
		Suizenjina	*Kinjiso, handama*	Crops whose stems and leaves are harvested
		Zucchini		Crops whose fruits are harvested
		Zucchini (flower)	*Hanazucchini*	Crops whose flowers are harvested
		Great (edible) burdock	Salsyfy, *baramonshin*	Crops whose roots are harvested
		Senega	*Hirohasenega*	Crops whose roots are harvested
		Celeriac	Root celery	
		Cnidium rhizome		Crops whose rhizomes are harvested
		Japanese green gentian		Crops whose whole grasses are harvested
		Japanese royal fern		Crops whose leaves are harvested
		Rhubarb	*Shinshu-daio*	Crops whose rhizomes are harvested

		Japanese radish(*daikon*)	*Hadaikon, daikonna*	Crops whose stems and leaves, and roots are harvested
		Bamboo	*Madake, mosochiku, hachiku*	Crops whose young poles are harvested
		Onion		Crops whose bulbs are harvested
		Tarragon	Estragon, french tarragon, russian tarragon	Crops whose stems and leaves are harvested
		Japanese angelica-tree	*Taranome*	Crops whose shoots are harvested
		Chicory	*Kikunigana*	Crops whose stems and leaves (softened buds) are harvested
		Chicory （stump）	*Kikunigana*	Crops whose roots are harvested
		Chive	*Ezonegi, seiyo-asatsuki, siburetto*	Crops whose leaves are harvested
		Japanese artichoke		Crops whose tubers are harvested
		New Zealand spinach	*Hamana, hamajisha*	Crops whose stems and leaves are harvested
		Indian spinach	*Shintsurumurasaki*	
		Japanese silver leaf		Crops whose leaf stalks are harvested
		Red pepper (leaf)		Crops whose leaves are harvested
		Japanese angelica root		Crops whose rhizomes are harvested
		Tosukebofu		Crops whose roots are harvested
		Dokudami		Crops whose whole grasses above ground are harvested
		Gutta-percha tree （leaf）		Crops whose leaves are harvested
		Tomato		Crops whose fruits are harvested, not including those of 3cm or less in diameter
		Aconite (for medical use)		Crops whose root tubers are harvested
		Trevise	*Torebitsu*	Crops whose stems and leaves after headed up are harvested
		Eggplant		Crops whose fruits harvested
		Shepherd's-purse		Crops whose stems and leaves are harvested. One of the seven spring plants

		Nandin (leaf)		Crops whose leaves are harvested
		Balsam pear	*Tsurureishi*	Crops whose fruits are harvested
		Chinese chives	*Kinira*	Crops whose leaves are harvested
		Chinese chives (scape)	*Hananira*	Crops whose flower buds and scapes are harvested
		Carrot	*Kintoki-ninjin, seiyo-ninjin, shima-ninjin*	Crops whose roots are harvested
		Garlic	Jumbo-*ninniku*, elephant garlic, greathead garlic	Crops whose bulbs are harvested
		Garlic (scape)		Crops whose scapes are harvested
		Welsh onion	*Kujho-negi, kaga-futonegi, senju-negi, yagura-negi, shimonita-negi, riiki*	Crops whose stems and leaves are harvested
		Alium grayi		Crops whose leaves and bulbs are harvested
		Chinese cabbage(*brassica rapa* var. *Pekinensis*)		Crops whose stems and leaves after headed up are harvested
		Chickweed	*Kohakobe, midori-hakobe*	Crops whose stems and leaves are harvested
		Hagobo		Crops whose comparatively young leaves are harvested with roots
		Hashoga	*Yanaka-shoga, hajikami-shoga, fude-shoga*	Crops whose young rhizomes are harvested with living leaves
		Hasuimo (leaf stalk)		Crops whose leaf stalks are harvested
		Hatamanegi	*Hatamanegi*	Leaves and bulbs of onion at their comparatively young stages (when the bulbs are starting to grow larger) to be harvested
		Hatawasabi	*Okawasabi*	Crops whose leaves, scapes, rhizomes, and roots are harvested. Those cultivated in dry fields.
		Hatawasabi (leaf)	*Hawasabi*	Crops whose leaves are harvested Those cultivated in dry fields.
		Hatsukadaikon	*Hatsukadaikon,* radish, *hatsukadaikon*	Crops whose stems and leaves, and roots are harvested
		Hana-okura		Crops whose flowers are harvested
		Vanilla		Crops whose fruits are harvested

		Haninniku		Leaves and bulbs of garlic at its comparatively young stages to be harvested
		Cudweed	*Gogyo, ogyo*	Crops whose stems and leaves are harvested. One of the seven spring plants.
		Coffee senna (stem and leaf)		Crops whose whole grasses above ground are harvested
		Hamabofu (leaf)		Crops whose leaves are harvested
		Bell pepper	*Oojishi*, california wonder, color-*piman*, *oranda*-paprika	Crops whose fruits are harvested
		Hikiokoshi		Crops whose whole grasses above ground are harvested
		Non-heading brussels sprouts	Petit veil, mini veil	Crops whose stems and leaves, and axillary buds are harvested
		Loquat (leaf)		Crops whose leaves are harvested
		Japanese butterbur		Crops whose leaf stalks are harvested
		Japanese butterbur (flower bud)	*Fukinoto*	Flower buds of Japanese butterbur to be harvested
		Leaf beet	Betterave, *akafudanso*, Swiss chard, detroit	Crops whose leaves are harvested
		Florence fennel		Crops whose leaf stalks are harvested for their enlarged basal part
		Broccoli		Crops whose flower buds and scapes are harvested
		Summer cypress	*Tonburi, hokigusa*	Crops whose utricle are harvested
		Spinach		Crops whose stems and leaves are harvested
		Hosobawadan	*Nigana, njana*	
		Botanbofu	*Choomeiso, sakuna, chomiigusa*	
		Borage	*Rurijisa*	Crops whose stems and leaves, and flowers are harvested
		Corn salad	Corn salad, marsh lettuce	Crops whose stems and leaves are harvested
		Oriental melon		Crops whose mature fruits are harvested
		Manchurian wild rice	*Makomo*	Crops whose stems are harvested for

				their parts enlarged by parasites of fungus *Ustilago esculenta*
		Sweet marjoram	Sweet marjoram, pot marjoram, golden marjoram, mayorana	Crops whose stems and leaves are harvested
		Mishimasaiko		Crops whose rhizomes are harvested
		Mizuimo (leaf stalk)	*Taimo*	Crops whose leaf stalks are harvested
		Cherry tomato		Crops whose fruits are harvested. Those of 3cm or less in diameter
		Japanese ginger (stem and leaf)	*Myogatake*	Crops whose stems and leaves after softened are harvested
		Japanese ginger (spike)	*Hanamyoga*	Crops whose spikes are harvested
		Purple turmeric	*Gajutsu*	Crops whose stems and leaves are harvested
		Brussels sprouts	*Mekyabetsu, komochikanran*	Crops whose axillary buds after headed up are harvested
		Melon	*Ares melon, ams melon, andes melon, elizabeth melon, kinshomelon, kinshomelon, quincy melon, takami melon, honey dew melon, papaya melon, prince melon, hamiuri*	Crops whose mature fruits are harvested
		Momijigasa	*Shidoke, momijikusa, taikona, toukichina*	Crops whose stems and leaves are harvested
		Moriazami	*Yamagobo, goboazami*	Crops whose roots are harvested
		Malta jute	*Taiwantsunaso, shimatsunaso*	Crops whose stems and leaves are harvested
		Ginseng for medical use	*Otaneninjin, chosen-ninjin, korai-ninjin*	Crops whose roots are harvested
		Yacon (stem and leaf)		Crops whose stems and leaves are harvested
		Water pepper	*Ayutade, hontade, matade*	
		Japanese yam (*Dioscorea japonica*) (brood	*Nagaimo-no-mukago, yamanoimo(jinenjo)-no-mukago*	Crops whose brood buds are harvested

		bud)		
		Young corn	Baby corn	Young fruits (maize ears) of corn to be harvested
		Strawberry saxifrage		Crops whose leaves are harvested
		Yobusumaso	*Bonna*	Crops whose stems and leaves are harvested
		Aster *yomena*	*Hagina*	
		Mugwort		
		Rakkyo	*Rakkyo* (eschalot cultivation)	Crops whose bulbs are harvested
		Rhubarb	Edible daio	Crops whose leaf stalks are harvested
		Lettuce	Crisp-head type *tamachisha*	Crops whose stems and leaves after headed up are harvested
		Lemongrass	Melissa glass, lemon gaya, lemon so, fever glass	Crops whose stems and leaves are harvested
		Lemon verbena	*Kosuiboku*	Crops whose stems and leaves are harvested
		Lotus root	*Hasu*	Crops whose rhizomes are harvested
		Wakegi		Crops whose leaves and bulbs are harvested
		Wasabi	*Mizuwasabi*	Crops whose leaves, scapes, rhizomes, and roots are harvested. Those cultivated in a water system
		Wasabi daikon	Horseradish, *seiyo-wasabi*	Crops whose roots are harvested
		Bracken		Crops whose leaves are harvested
Mushrooms		*Enokitake*		Crops whose fruit bodies (mushroom) are harvested
		King oyster mushroom	*Kaorihiratake*	
		Shiitake		
		Nameko		
		Oyster mushroom		
		Bunashimeji		

		mushroom		
		Hen of the woods		
		Mushroom	*Tsukuritake*	
Tuber crops		Ground nut	Apios	Crops whose root tubers are harvested
		Sweet potato	*Satsumaimo*, *shimon-imo*	
		Jerusalem artichoke	*Butaimo*	Crops whose tubers are harvested
		Konjak (konnyaku)		
		Taro(*colocasia esculenta*)	*ebiimo, takenokoimo, yatsugashira*	
		Hasuimo (tuber)		
		White potato	*Jagaimo*	
		Mizuimo	*Taimo*	
		Yacon		Crops whose root tubers are harvested
		Japanese yam (*Dioscorea japonica*)	*Yamatoimo, jinenjo, maruimo, nagaimo, tokkuriimo, iseimo, ichoimo, tsukuneimo*, *daijo*	Crops whose tubers are harvested
Pulses (seeds)		*Adzuki* bean	*dainagon*	Crops whose mature seeds are harvested
		String bean(*phaseolus vulgaris*)	*Ingen, kintokimame, toramame,uzuramame*	
		Pea		
		Cowpea		
		Fava bean		
		Soybean		
		Sword bean		
		Hyacinth bean	*Sengokumame, kagatsurumame, turumame*	
		Scarlet runner	*Hanamame*	
		Peanut	*Nankinmame*, *rakkasei*	
		Egoma (*perilla frutescens* var. *Frutescens*) (seed)		Crops whose seeds are harvested
		Oriental senna (seed)	*Rokkakuso*	
		Caraway（fruit）	Caraway	Crops whose fruits are harvested
		Indian mustard (seed)		Crops whose seeds are harvested
		Sesame		Crops whose seeds

				are harvested
		Coriander(fruit)	*Coendoro*	Crops whose fruits are harvested
		Sugar cane		Crops whose stems are harvested to gather sugar
		Shiso(perilla frutescens var. Crispa)(seed)		Crops whose seeds are harvested
		Edible flax		
		Edible safflower (seed)		
		Tea		Crops whose shoots are harvested
		Dill (seed)		Crops whose seeds are harvested
		Sugar beet	*Satodaikon*	Crops whose roots are harvested to gather sugar
		Rapeseed		Crops whose seeds are harvested
		Coffee senna		
		Water chestnut		
		Sunflower (seed)		Crops whose seeds are harvested
		Fennel (seed)	Uikyo(seed)	
		Hop	*Seiyo-karahanaso*	Crops whose female flower spikes are harvested
Forage crops	Pasture grass	Pasture grass of the grass family	Orchard grass, timothy, italian ryegrass, thor fescue, perrenial rye grass, bahiha grass	Crops whose stems and leaves are harvested for animal feed
		Pasture grass of the pea family	Red clover, white clover, alfalfa	
		Oats for animal feed		
		Corn for animal feed		Crops whose stems and leaves, and female maize ears are harvested for animal feed
		Sorghum	Sudan grass	Crops whose stems and leaves are harvested for animal feed

(Note 1)
Kyoho type tetraploid varieties of grapes:
Kyoho, , pione., Aki queen, Fujiminori, Sunny rouge, Suiho,Kokuo, Gorby, Shigyoku, Shinano smile, Takatsuma, Tama Yutaka, Hakuho, Beniyoshi, Izunishiki, Izumo queen, Ichiki marl, Uehara 540, aurora black, Olympia, Sagami, jasmine, dark ridge, Takazumi, High Bailey, honey black, honey Venus, BlackOpus, black Olympia, Beniizu, Benizuiho, Benifuji,Beniyamabiko, Ryuho, red queen, road berry, Ogyoku, Tenshu, etc.

Diploid American varieties of grapes:
Adirondac, Muscat berry A, Buffalo (EarlySteuben), Niagara Marabelfa, Urubana, Campbell,

Campbell Early, stew Ben, say bell 9110, Seneca, Dayulu, Tano Red, Tabiji, Niagara, Benikanazawa, Benishioya, Beninanyo, Portland, Red port, Peerless, New York Muscat, North black, North read, Violet Uehara, Fredonia, Himrod Seedless

Diploid European varieties of grapes:
Seto Giants, Rosaki, Mario, Rosary Bianco, Ruby Okuyama, Muscat of Alexandria, Shine Muscat, CG88435, Alphonse lavallee, Italia, *Kaiotome,* Kaiji, KattaKourgan, Cabernet Sauvignon, green summer, Kurugan rose, Konigin der Weingarten, Koshu, Koshu Sanjaku, Gold, Goldfinger, Zabalkanski, Citronelle, Chardonnay, Akamine, Cecilia, Zhana, Cherry, Jingzaojing, Nyunai, neo-Mart, neo-Muscat, Nehelescol, Baladi, Venus、Pizzutello Bianco、Black Swan、Black Sanjaku、Flame Tokay、Beijiagan、Beni Alexandria, Benisanjaku, Benitamaki, Beni Pizzutello, Marnaizi, Muscat Kofu, Muscat Duke Amore, Muscat
Hamburg, Muscat Violet, manicure finger, Morghen Shane, Yatomi Rosa, unicorn, Rizamat, RichBaba, Ryugan、 Ryogyoku, Rubel Muscat, Ruby Okubo, red glove, Red Nehelescol, Royal, Rosario　Rosso, Arisa, Oka, Shien, Hilo Hamburg, etc.

Triploid varieties of grapes:
King Dela, Summer Black, *Kaimirei,* Nagano Purple, Aki Seedless, Mirei, etc.

(Note 2)
The large crop groups or the medium crop groups other than “small-grain cereals of the Poaceae”, “small stone fruits”, “berries”, “cucurbitaceous fruit (to be used for pickles)”, “*nabanas*”, “non-head brassica leafy vegetables”, “non-head lettuces”, “leafy vegetables of the Lamiaceae”, and “leafy vegetables of the Apiaceae”, shall include crops other than those listed in the “Crop name” column to be included in the relevant group, if they belong to the relevant crop group.

(Appendix Table1-2)

Applicable crops (Crops other than those that are to be used for food (including industrial crops and crops to be used for animal feed): Crops for which test results regarding residue in crops are not required.)

Large crop group name	Medium crop group name	Crop name	Examples of alias name, local name, variety name, etc. included in the crop name	Remarks
Flowering plants and ornamental foliage plants		Iceland poppy		
		Ivy geranium		
		Iris		
		Agapanthus		
		Ageratum		
		Morning glory		
		Japanese thistle		
		Maidenhair		
		Ajuga		
		Aster		
		Astilbe		
		Rose grass		
		Ananas		
		Poppy anemone		
		Amazon lily		
		Scented solomon's seal		
		Blue daze		
		Onion		
		Peruvian lily		

Aloe		
Anthurium		
Busy lizzie		
Nepenthes		
Persian violet		
Eremurus		
Okamezasa		
Odontoglossum		
Ominaeshi		
Japanese rohdea		
Oncidium		
Carnation		
Gerbera		
Gazania		
Common gypsophila		
Cattleya		
Calla		
Caladium		
Kalanchoe		
Slipperflower		
Kawaraketsumei		
Kangaroo-paw		
Ornamental asparagus		
Ornamental eggplant		
Ornamental mosochiku		
Campanula		
Bellflower		
Chrysanthemum		
Snapdragon		
Common marigold		
Gladiolus		
Chrysanthemum		
Christmas rose		
Curcuma		
Clematis		
Gloxinia		
Crocus		
Gloriosa		
Cockscomb		
Shell flower		
Honey euryops		
Common cosmos		
Phalaenopsis		
Farewell-to-spring		
Common coleus		
Coreopsis		
Primrose		
Saponaria		
Scarlet sage		
Lobelia		
Sansevieria		
Sandersonia		
Bolivian sunset		
Persian cyclamen		

Cineraria		
Moss phlox		
Chinese peony		
Japanese anemone		
Perennial aster		
Perennial baby's breath		
Perennial statice		
Cymbidium		
Narcissus		
Sweet pea		
Statice		
Garden stock		
Streptocarpus		
Strelitzia		
Sunagoke		
Peace lily		
Speed lyon		
Violet		
Garden geranium		
Saintpaulia		
Globe amaranth		
Solidago		
Solidaster		
Taniwatari		
Dahlia		
Saxifrage		
Tuberose		
Tulip		
Greater periwinkle		
Disa		
Dumb cane		
Dimorphotheca		
Daisy		
Rocket larkspur		
Dendrobium		
Christmas cactus		
Aconite		
Lisianthus		
Bluewings		
Garden nasturtium		
Pink		
Cup flower		
Love-in-a-mist		
Madagascar periwinkle		
Baby-blue-eyes		
Nolana		
Verbena		
Joseph's coat		
Bacopa		
Japanese iris		
Hana-tori-kabuto		
Hanahasu		
Carifornia poppy		
Ornamental kale		

Rose		
Pansy		
Vanda		
Blackberry lily		
Hypoestes		
Sunflower		
Osbeckia chinensis		
Helianthus cucumerifolius		
Common zinnia		
Hyacinth		
Creeping fig		
Variegated scented solomon's seal		
Philodendron		
Cape plumbago		
Bupleurum		
Bridal veil		
Swan river daisy		
Freesia		
Primrose		
Blue scarlet sage		
Blue star		
Blue daisy		
Blue race flower		
Phlox		
Browallia		
Begonia		
Petunia		
English ivy		
Safflower		
Baby rose		
Pelargonium		
Strawflower		
Heloconia		
Veronica		
Garden balsam		
Chinese lantern plant		
Portulaca		
Tree peony		
Pothos		
White race flower		
Marguerite		
Garden portulaca		
Matricaria		
Marigold		
Monkey flower		
Miyakowasure (miyamayomena)		
Miltonia		
Muscari		
Monstera		
Cornflower		
New york aster		
Euphorbia fulgens		
Lily		

		Gray-leaved euryops		
		Larkspur		
		Rice flower		
		Turban buttercup		
		Lavatera		
		Lavender		
		Gay feather		
		Lysimachia		
		Limnanthes		
		Gentian		
		Cone flower		
		Lupine		
		Leatherleaf fern		
		Red ginger		
		Lotus flower		
		Laurentia		
		Rochea		
		Edging lobelia		
		Forget-me-not		
		Great burnet		
Trees and shrubs	azaleas	Azalea		
		Omurasaki		
		Kurume azalea		
		Satsuki azalea		
		Rhododendron		
	camelias	Sazanqua		
		Camellia reticulata		
		Wild camellia		
		Snow camellia		
		Acacia		
		Japanese hydrangea		
		Luculia		
		Zebra plant		
		Blue japanese oak		
		False aralia		
		Areca palm		
		Japanese podocarp		
		Quercus phillyraeoides		
		Japanese winterberry		
		Erica		
		Ogonkujakuhiba		
		Kakuremino		
		Malayan banyan		
		Japanese photinia		
		Carolina jasmine		
		Lady palm		
		Ivy		
		Scarlet bottlebrush		
		Cape jasmine		
		Firecracker flower		
		Croton		
		Orange jessamine		
		Keyaki		
		Curly palm		
		Umbrella pine		

		Monterey cypress		
		Reeves spirea		
		Cotoneaster		
		Indian rubber tree		
		Cabbage tree		
		Konronka		
		Cleyera japonica		
		Japanese cherry		
		Crape myrtle		
		Sweet viburnum		
		Siberian dogwoood		
		Hawthorn		
		Japanese cornel		
		Chinese ixora		
		Schefflera		
		Japanese anise-tree		
		Jacaranda		
		Jasminum polyanthum		
		Yeddo hawthorn		
		Shirakashi		
		Winter daphne		
		Japanese cedar		
		Doghobble		
		Cherry laurel		
		Sennryo		
		Chosenmaki		
		Japanese box		
		Parlor palm		
		Pigeon-berry		
		Bottle palm		
		Tobira		
		Dracaena		
		Mountain ash		
		Nandin		
		Winged spindle		
		Great trumpet creeper		
		Melastoma		
		Hibiscus		
		Creeping chinese juniper		
		French peanut		
		Flowering dogwood		
		Japanese mahonia		
		Eurya		
		Hinoki cypress Japanese cypress		
		Hypericum		
		Bougainvillea		
		Japanese spurge		
		Bouvardia		
		Weeping fig		
		Poinsettia		
		Fuchsia		
		Polyscias		
		Red boronia		
		Evergreen spindle		

		Japanese witch hazel		
		Mandevilla		
		Table dogwood		
		Sweet osmanthus		
		Lily magnolia		
		Mokkoku		
		Willow		
		Yabusanzashi		
		Eucalyptus		
		Thunberg's meadowsweet		
		Yucca		
		Lantana		
		Leadwort		
		Weeping forsythia		
		Rush		
		Chinese mat-grass		
		Tobacco		
		Flax		
Lawn grasses	Western lawn grasses	Western lawn grass (orchard grass)		
		Western lawn grass (kentucky bluegrass)		
		Western lawn grass (tifton		
		Western lawn grass (barmuda grass)		
		Western lawn grass (fescue)		
		Western lawn grass (bluegrass)		
		Western lawn grass (perennial ryegrass)		
		Western lawn grass (bent grass)		
		Western lawn grass (rye grass)		
	Japanese lawn grasses	Japanese lawn grass (korean lawn grass)		
		Japanese lawn grass (*himekoraishiba*)		
		Japanese lawn grass (*noshiba*)		
		Mulberry		

(Note 1)
The large crop groups and medium crop groups shall include crops other than those listed in the "Crop name" column to be included in the relevant group, if they belong to the relevant crop group.

(Appendix Table 2)

○ The required number of trials for each crop group

Crop group name	The required number of trials regarding efficacy test and phytotoxicity test	The required number of trials regarding limit test for phytotoxicity
Cereal grains	A total of 6 or more trials on crops included in the relevant crop group (Trials shall be conducted over two years.)	A total of 4 or more trials on 2 or more types of crops included in the relevant crop group
Cereals(others)	A total of 6 or more trials, including: 2 or more trials on sweet corn, and 4 or more trials on 2 or more other types of crops included in the relevant crop group	A total of 6 or more trials on 3 or more types of crops that were used for efficacy test and phytotoxicity test as test crop
Small-grain cereals of the Poaceae	A total of 4 or more trials on 2 or more types of crops included in the relevant crop group	A total of 4 or more trials on 2 or more types of crops that were used for efficacy test and phytotoxicity test as test crop
Citrus fruits	A total of 6 or more trials on crops included in the relevant crop group (Trials shall be conducted over two years)	A total of 4 or more trials, including: 2 or more trials on mandarin orange, and 2 or more trials on 1 or more other types of crops included in the relevant crop group
Small stone fruits	A total of 6 or more trials on crops included in the relevant crop group	A total of 4 or more trials on 2 or more types of crops included in the relevant crop group
Berries	A total of 6 or more trials on 3 or more types of crops included in the relevant crop group	A total of 6 or more trials on 3 or more types of crops that were used for efficacy test and phytotoxicity test as test crop
Cucurbitaceous fruit (to be used for pickles)	A total of 6 or more trials on 3 or more types of crops included in vegetables of the Cucurbitaceae (limited to cases where they include 1 or more type of crops included in the relevant crop group)	A total of 6 or more trials on 3 or more types of crops included in the relevant crop group
Chili peppers	A total of 6 or more trials on crops included in the relevant crop group, or bell pepper	A total of 2 or more trials on crops included in the relevant crop group, or bell pepper
Nabanas	A total of 6 or more trials on 3 or more types of crops included in "non-head brassica leafy vegetables" or "*nabana*s"	A total of 6 or more trials on 3 or more types of crops that were used for efficacy test and phytotoxicity test as test crop
Non-head brassica leafy vegetables	A total of 6 or more trials on 3 or more types of crops included in "non-head brassica leafy vegetables" or "*nabana*s"	A total of 6 or more trials with 3 or more types of crops that were used for efficacy test and phytotoxicity test as test crop

Non-head lettuces	A total of 6 or more trials on crops included in the relevant crop group, or lettuce	A total of 2 or more trials on 1 or more types of crops included in the relevant crop group
Leafy vegetables of the Lamiaceae	A total of 6 or more trials, including: 2 or more trials on *shiso*(*Perilla frutescens* var. *crispa*), and 4 or more trials on 2 or more types of crops included in the relevant crop group	A total of 6 or more trials on 3 or more types of crops that were used for efficacy test and phytotoxicity test as test crop
Leafy vegetables of the Apiaceae	A total of 6 or more trials, including: 2 or more trials on celery, and 4 or more trials on 2 or more types of crops included in the relevant crop group	A total of 6 or more trials on 3 or more types of crops that were used for efficacy test and phytotoxicity test as test crop
Legume vegetables	A total of 6 or more trials on 3 or more types of crops included in "pulses (seeds)" or "legume vegetables"	A total of 6 or more trials on 3 or more types of crops that were used for efficacy test and phytotoxicity test as test crop
Pulses(seeds)	A total of 6 or more trials on 3 or more types of crops included in "pulses (seeds)" or "legume vegetables "	A total of 6 or more trials on 3 or more types of crops that were used for efficacy test and phytotoxicity test as test crop
Mushrooms	A total of 6 or more trials on 3 or more types of crops included in the relevant crop group	A total of 6 or more trials on 3 or more types of crops that were used for efficacy test and phytotoxicity test as test crop
Tuber crops	A total of 6 or more trials on 3 or more types of crops included in the relevant crop group	A total of 6 or more trials on 3 or more types of crops that were used for efficacy test and phytotoxicity test as test crop
Flowering plants and ornamental foliage plants	A total of 6 or more trials on 3 or more types of crops included in the relevant crop group	A total of 6 or more trials on 3 or more types of crops that were used for efficacy test and phytotoxicity test as test crop
Trees and shrubs	A total of 6 or more trials on 3 or more types of crops included in the relevant crop group	A total of 6 or more trials on 3 or more types of crops that were used for efficacy test and phytotoxicity test as test crop

(Note 1) Efficacy test and phytotoxicity test shall be conducted for each combination of applicable diseases or insect pests and the application method.

(Note 2) Limit test for phytotoxicity shall be conducted for each combination of the application timing and the application method.

(Note 3) The number of trials for each test crop shall be 2 or more.

(Appendix Table 3-1)

○ Crops whose production volume is particularly high

Crops to be used for food (including industrial crops and crops to be used for animal feed): Rice (paddy rice and upland rice), wheat, mandarin orange, Japanese persimmon, pear (Japanese pear and European pear), apple, cabbage, cucumber, watermelon, Japanese radish(*daikon*), onion, tomato, eggplant, carrot, welsh onion, Chinese cabbage(*Brassica rapa* var. *pekinensis*), spinach, lettuce, sweet potato, potato, soybean, tea, pasture grass of the grass family, pasture grass of the pea family, corn to be used for animal feed and sorghum

(Appendix Table 3-2)

○Crops whose production volume is high

Crops to be used for food (including industrial crops and crops to be used for animal feed): Barley, sweet corn, iyokan(*Citrus iyo*), shiranui, natsumikan(*Citrus natsudaidai*), hassaku(*Citrus hassaku*), Japanese apricot(*Prunus mume*), kiwifruit, grape, peach, komatsuna(*Brassica rapa* var. *perviridis*), qinggengcai(*Brassica rapa* var. *chinensis*), nozawana(*Brassica rapa* L. var. *hakabura*), edamame(immature soybeans in the pod), common bean(pods and immature seed), celery, strawberry, turnip, squash, burdock, garland chrysanthemum, ginger, Chinese chives, bell pepper, broccoli, cherry tomato, melon, lotus root, konjac(*Amorphophallus konjac*), taro (*Colocasia esculenta*), Japanese yam(*Dioscorea japonica*), azuki bean, sugar cane, sugar beet and oats to be used for animal feed
Crops other than those to be used for food: Chrysanthemum and lawn grasses

(Appendix Table 4)

○ Crop group name and test crop

Crop group name	Test crop
Cereal grains	Wheat and barley
Small-grain cereals of the Poaceae	Foxtail millet
Citrus fruits	Mandarin orange, one large-fruit type and one small-fruit type (e.g. *kabosu, sudachi,* etc.) However, in cases where the relevant agricultural chemical is not directly applied to crops such as in the case of pesticides for soil treatment or herbicides, etc. and the residue level in either mandarin orange or one large-fruit type is equal to or lower than the limit of quantification (the limit of detection), the test results on the said fruit may substitute for the relevant test results.
Small stone fruits	Japanese apricot (*Prunus mume*) and one different type of crop included in the relevant crop group
Berries	One type of crop from each of the Ericaceae, Rosaceae and Saxifragaceae, that are included in the relevant crop group

Cucurbitaceous fruit (to be used for pickles)	Oriental pickling melon (*Cucumis melo* ver. *conomon*) and one different type of crop included in the relevant crop group
Chili peppers	*Shishito* pepper and one different type of crop included in the relevant crop group However, in cases where the relevant agricultural chemical is not directly applied to crops such as in the case of pesticides for soil treatment, herbicides, etc. and the residue level in bell pepper is equal to or lower than the limit of quantification(the limit of detection), the test results on bell pepper may substitute for the relevant test results.
Nabanas	2 types of crop included in the relevant crop group
Non-head brassica leafy vegetables	*Komatsuna*(*Brassica rapa* var. *perviridis*), *Mizuna* (*Brassica rapa* var. *nipposinica*) and one different type of crop included in the relevant crop group
Non-head lettuces	2 types of crops included in the relevant crop group. However, in cases where the relevant agricultural chemical is not directly applied to crops such as in the case of pesticides for soil treatment, herbicides, etc. and the residue level in lettuce is equal to or lower than the limit of quantification(the limit of detection), the test results on lettuce may substitute for the relevant test results.
Legume vegetables	*Edamame*(immature soybeans in the pod), podded pea and common bean(pods and immature seed)
Mushrooms	*Shiitake* and one different type of crop included in the relevant crop group
Leafy vegetables of the Lamiaceae	*Shiso*(*Perilla frutescens* var. *crispa*) or sage(*Salvia officinalis*) or mint and one different type of crop included in the relevant crop group
Leafy vegetables of the Apiaceae	*Kinsai* or coriander (leaf) or *mitsuba*(Japanese honewort) and one different type of crop included in the relevant crop group
Pulses(seeds)	Soybean, peanut and one different type of crop included in the relevant crop group. However, in cases where the applicable crops do not include peanut, the test crops shall be soybean and one type of crop other than peanut included in the relevant crop group.

(Appendix Table 5)

○ Crop group name and test crop to be applied in test on residue in crops when the residue of the relevant agricultural chemical is not detected or is extremely low level in the applicable crops due to the nature of the relevant agricultural chemical and its application method.

Crop group name	Test crop
Cereals (others)	2 or more types of crops belonging to different families included in "cereals (others)".
Fruit trees	3 or more types of crops belonging to different families included in "fruit trees".
Vegetables	5 or more types of crops belonging to different families included in "vegetables".
Tuber crops	3 or more types of crops belonging to different families included in "tuber crops".
Pulses (seeds)	Peanut and one different type of crops included in "pulses (seeds)". However, in cases where the applicable crops do not include peanut, the test crop shall be one type of crop other than peanut included in "pulses(seeds)".

(Appendix Table 6)

○ Test crops for each applicable crop

Crop name	Test crop
Corn	Maize and sweet corn
Japanese plum	Japanese plum or nectarine
Nectarine	Nectarine or Japanese plum; However, if the applicant submits test results of 3 or more trials on peach, such test results may substitute for the relevant test results.
Grape	One small fruit type and one large fruit type (Note 1). However, in cases where the relevant agricultural chemical is not directly applied to crops such as in the case of pesticides for soil treatment, herbicides, etc. and the residue level in grape is equal to or lower than the limit of quantification (the limit of detection), the test results of 2 trials on one crop regardless of small fruit type or large fruit type may substitute for the relevant test results.
Pear (Note 2)	European pear, Japanese pear or Chinese pear
Asatsuki(*Allium schoenoprasum* var. *foliosum*)	*Asatsuki*(*Allium schoenoprasum* var. *foliosum*) However, in cases where the relevant agricultural chemical is not directly applied to crops such as in the case of pesticides for soil treatment, herbicides, etc. and the residue level in both *hanegi* and *nebukanegi* is equal to or lower than the limit of quantification(the limit of detection), the test results on *hanegi* and *nebukanegi* may substitute for the relevant test results.
Cauliflower	Cauliflower; However, if the applicant submits test results of 3

	or more trials on broccoli, such test results may substitute for the relevant test results.
Tomato (Note 3)	Tomato or cherry tomato
Welsh onion	*Hanegi* and *nebukanegi* (Note 4)
Oriental melon	Oriental melon; However, if the applicant submits test results of 3 or more trials on melon, such test results may substitute for the relevant test results.
Peas(succulent seed)	Peas(succulent seed)or podded pea
Cherry tomato (Note 3)	Cherry tomato. However, in cases where the relevant agricultural chemical is not directly applied to crops such as in the case of pesticides for soil treatment, herbicides, etc. and the residue level in tomato is equal to or lower than the limit of quantification(the limit of detection), the test results on tomato may substitute for the relevant test results.
Wakegi	*Wakegi*. However, in cases where the relevant agricultural chemical is not directly applied to crops such as in the case of pesticides for soil treatment, herbicides, etc. and the residue level in both *hanegi* and *nebukanegi* is equal to or lower than the limit of quantification(the limit of detection), the test results on *hanegi* and *nebukanegi* may substitute for the relevant test results.
Zucchini	Zucchini or cucumber
Sweet potato	Sweet potato; However, in cases where it is considered that the relevant agricultural chemical does not remain in the harvested product in light of its application method, etc. and the residue level in potato is less than the limit of quantification on the basis of test results of 6 or more trials on potato, the test results on potato may substitute for the relevant test results.
Taro (*Colocasia esculenta*)	Taro(*Colocasia esculenta*); However, in cases where it is considered that the relevant agricultural chemical does not remain in the harvested product in light of its application method, etc. and the residue level in sweet potato is less than the limit of quantification on the basis of test results of 6 or more trials on sweet potato or the residue level in potato is less than the limit of quantification on the basis of test results of 6 or more trials on potato, the test results on sweet potato or on potato may substitute for the relevant test results.
Potato	Potato; However, in cases where it is considered that the relevant agricultural chemical does not remain in the harvested product in light of its application method, etc. and the residue level in potato is less than the limit of quantification on the basis of test results of 6 or more trials on sweet potato, the test results on sweet potato may substitute for the relevant test results.
Japanese yam (*Dioscorea japonica*)	Japanese yam (*Dioscorea japonica*); However, in cases where it is considered that the relevant agricultural chemical does not remain in the harvested product in light of its application method, etc. and the residue level in sweet potato is less than the limit of quantification on the basis of test results of 6 or more trials on sweet potato or the residue level in potato is less than the limit of quantification on the basis of test results of 6 or more trials on potato, the test results on sweet potato or on potato may substitute for the relevant test results.

(Note 1):
Test shall be conducted on three or more trials including both small fruit type and large fruit type, and two or more trials among them shall be from the type with higher residues. "Small fruit type" shall refer to grapes whose weight per berry is more or less 1.5 g such as Delaware, while "large fruit type" shall refer to grapes other than this.

(Note 2):
"Pear" shall refer to European pear, Japanese pear and Chinese pear.

(Note 3):
"Cherry tomato" shall refer to tomatoes whose diameter is not more than 3 cm, while "tomato" shall refer to tomatoes other than this.

(Note 4):
Test shall be conducted on three trials on each of *hanegi* and *nebukanegi* .

Appended Format 1

Analytical Result Report of Water Pollution
(Paddy Field Water or Percolation Water)

Date:

1. Staffer in charge of Analysis (Section/Name) _____ Name of Sponsor: _____
2. Name of Test Substance _____ Formulation: _____
3. Chemical Name of Active Ingredients _____ Applied Concentration/Dose _____
   and Percentage Content (%) of Test Substance _____ Sample Preparation Place _____
4. Analytical Object Substance (Name of Component): _____
5. Test Paddy Field (Scale: Vertical length x Horizontal Length) (m x m) _____ (Soil classification) Test Area 1 (Soil Texture) Test Area 1
   Test Area 2 Test Area 2
6. Gist of Analytical Method _____
   (Details in appended sheet) (Limit of determination) mg/L (Recovery) Average recovery by addition of mg/L 1. %,, 2. %
   Average recovery by addition of mg/L %, %
7. Analytical results:

Test Area	Sample Active Ingredients dose	Date of treatment	Date of sampling	Date of Sending sample	Date of Arrival of sample	No. of treatments	No. of Secular days	No. of analyses	Concentration (mg/L)			Date of Analyses of Samples	Remarks (Sample preservation method/storing period, etc.)
									Analytical value	R	Measurement value		
Test Area1	–	–	/ /	/ /	/ /	0	–	2				/ /	Measurement value
	(g/10g)	/ /	/	/	/	1	0(*)	2				/	
	-		/	/	/	1	1	2				/	
			/	/	/	1	3	2				/	
			/	/	/	1	7	2				/	
			/	/	/	1	14	2				/	
Test Area2	–	/ /	/ /	/ /	/ /	0	_	2				/ /	
	(g/10g)	/ /	/	/	/	1	0(*)	2				/	
			/	/	/	1	1	2				/	
			/	/	/	1	3	2				/	
			/	/	/	1	7	2				/	
			/	/	/	1	14	2				/	

Note: If stored until the analytical date, describe the storage condition.
(*) Time after treatment: Test area 1: _____ hour(s)
Test area 2: _____ hour(s)

Attached Sheet

I. Test Conditions

1. Test Substance
   Common name
   Formation
   Chemical name and percentage content
   Structural formula, and physicochemical quality of active ingredients
2. Structure of Test Paddy Field, etc.
   Scale
   Material
   Set place
   Structure and material of awning, beam permeability in U.V. area and measurement method, soil layer structure (including pebble, sand)
3. Characteristics of Test Soil
   Date of sampling
   Place of sampling
   Soil classification (gray lowland soil, gley soil, wet andosols, brown lowland soil, etc.)
   Matrix
   Soil Texture (classification as per USDA, etc.)
   Major clay minerals
   Organic carbon content (%)
   Soil pH (Water and KCl or $CaCl_2$)
   Cation ion exchange capacity (me/100g)
   Phosphoric acid absorption coefficient, etc.
   *: Omissible if described in a sample preparation specification.
4. Crop
   Name of crop
   Variety
5. Schedule of Test Implementation
   Summary of crop cultivation (date of plowing, date of transplant) and period of sampling samples, etc.
   Example: Plowing, plowing and irrigating the fields, transplant, test substance treatment, sampling period, etc.
6. Treated Concentration and Dose of Test Substance
   Treated dose of preparation per 10a
   Treated dose per a test area
7. Treating Method of Test Substance
   Date of treatment of test substance
   Treating method
8. Water Control Method of Test Paddy Field
   Water source, etc.
   Example: An underwater is supplied by a decreased quantity of water at O hr everyday, and a water depth is controlled at 5 cm.
9. Sample Sampling Method
   Sampling position, sampling tool, number of places, a quantity of water per one place, etc.
10. Measurement Methods of Meteorological Data, etc.
    Air temperature (the maximum, and the lowest), humidity, water temperature, measurement methods of pH, etc. of the paddy fields water
11. Others

III. Analytical Method

1. Indicator and equipment (Instrument)
2. Target Substance
   Reason for selecting analytical target substances, structural formula and physical/chemical quality
3. Operation Conditions of Analyzing Equipment
4. Preparation of Calibration Curve
5. Analyzing Operations
6. Limit of Determination and Recovery
   Average recovery and standard deviation percentage
7. Other Reviewing Items

IV. Test Results

1. Attenuation Curve in Test Paddy Field of Test Agricultural Chemical
2. Measurement Results of Data of Meteorology and Water Control, etc.
   Weather (fine, cloudy, rainy, etc.), air temperature (the maximum and the lowest), humidity, water temperature, quantity of supplied water, dropping and permeation, evaporated water quantity, water depth, pH in paddy fields, summary of crop cultivation, plant length of crop, etc.

V. List of Attached Referential Data

1. Table of Contents and Attached Diagrams of Gas chromatograph, etc. evidencing test reports
   (1) Chromatograms of one example of calibration curve, a detected example of a standard product, the minimum detected quantity or a confirmed example of limit of determination, an example of recovery, and analytical examples of untreated and treated samples, if analyzed with a gas chromatography and a high speed liquid chromatography.
   (2) Absorbed spectrum and absorbance of one example of calibration curve, a calibrated example of the standard product, conformed examples of the minimum detected quantity and limit of determination, an example of recovery, and analytical examples, etc. of untreated and treated samples, if analyzed with spectrometry.

Appended Format 2

Specification of Analyzed Sample Preparation of Water Pollution

(Date:)

1. Staffer in charge of sample preparation:_____
2. Sample preparation implementation place:_____
3. Test Paddy Field
   (1) Structure Scale (vertical length x horizontal length x thickness) and Material of Container _____
   Whether or not an awning facility is provided:_____
   structure of facility (height, etc.),:_____
   material of roof, light permeability in U.V. area and measurement method _____
   (2) Soil layer structure: test soil, sand, depth of pebbles, etc._____
   (3) Characteristics of soil
   Date of sampling:_____
   Place of sampling:_____
   Date of filling:_____
   Filling method:_____
   Soil group: (gray lowland soil, gley soil, high humid andosol, brown lowland soil, etc.)_____
   Soil texture classification: (clay soil, clay loam, light clay soil, loam, sand loam, etc.: classification as per USDA, etc.)_____
   Major clay minerals and mechanical composition:_____
   Organic carbon content_____%
   pH in soil_____(H_2O)_____(KCl or $CaCl_2$)_____
   Cation ion exchange capacity_____me/100g
   Phosphoric acid absorption coefficient_____
   Others_____
   (4) Set Place (Height of test paddy field from the ground, etc.)_____
   (5) Water supply method: Water supply source (tacit water, underwater, others)_____
   (6) Crop: Name of crop and variety_____
4. Summary of Crop Cultivation and Test Implementation Schedule
   Plowing, plowing and irrigating fields before transplanting rice seedlings, raising planting material, transplanting, kind of fertilizer application, quantity and period (date) _____
   _____
   _____
   _____

   Test substance treatment and sampling period (date) (weather at treatment: amount of rainfall, direction of wind, wind speed, etc.)_____
   _____
   _____
5. Test Substance
   (1) Common name:_____ Formulation:_____
   Chemical name and component content of Active Ingredients (%)
   _____(%)
   (2) Treating Concentration and amount (per 10a)_____
   (per test area)_____
   (3) Treatment method
   (entirely treated, treatment on water surface, soil treatment, etc.)_____
   _____
   (applied tool) _____
6. Method of sample preparation (equipment, etc used for sampling)_____
   Surface water of Paddy fields:
   (sampling equipment, etc.) _____
   (sampling position, water depth, etc.) _____
   Percolation water:
   (sampling device, method, etc.) _____
7. Agricultural chemicals used other than test substances (name of agricultural chemical, concentration, amount, date, others)_____
   _____

8. Table of Meteorology and Water Control, etc. (Test Area:)

Date																	
No . of secular days after treatment of test substance	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14		Average value
Air temperature (max. /min.)																	
Temperature (*1)																	
Weather (fine, cloudy, rainy, etc.)																	
Water temperature(*2)																	
Replenished water quantity (L)																	
Percolation water (L)																	
Dropping/permeation (cm)																	
Evaporated water amount																	
Water depth (*3) (cm)																	
Condition of test water (turbidity, etc.)																	
Plant length of crop (*4) (cm)																	
No. of Tilling																	
Others																	

(*1) Measurement time_____, (*2) Measurement time_____,
(*3) Only days on which the water is sampled, (*4) Only days on which the water is treated with an agricultural chemical.

9. Remarks:_____

Appended Format 3

Analytical Result Report of Residues in soil
(Paddy Field/Upland field)

Date:

1. Study director (Section/Name)_____ Name of sponsor: _____
2. Name of test substance:
(Describe the details in appended sheets 1 and 2) _____ (Formation) _____
3. Chemical name of active ingredient and percentage content (%) _____
_____
Applied concentration and usage of test substance _____
4. Analytical object substance (name of component) _____

Exemplification

5. Characteristics of test soil
(Describe the details in appended sheets 1 and 2)

Sample preparation place	1. ○○ Prefectural agricultural experimental station	(Origin of volcano)	Vocanic ash	(Soil texture)
	2. △△ Prefectural Agricultural Research Center		Alluvial	

_____

6. Gist of Analytical methods:
(Describe the details in Appended sheets 1 and 2)
(Extracting/refining method) _____
(Analyzing device) _____
(Operating conditions) _____
(Limit of determination) _____mg/kg
(Recovery) Average recovery by adding _____mg/kg _____%, _____%
Average recovery by adding _____mg/kg _____%, _____%

7. Analytical Results:

Test soil	Applied concentration and usage of test substance	Date of treatment	Date of sampling	Date of Sending sample	Date of Arrival of sample	No. of treatments	No. of Secular days	No. of analyses	Concentration (mg/L)			Date of Sample Analyses	No. of days of preservation	Remarks (Sample preservation method/storing period, etc.)
									Analytical value	R	Measurement value			
		/ /	/ /	/ /	/ /									

Attached Sheet 1

Soil Persistence Test Method

1.Test Substance

Specify the generic name, formulation, chemical name of active ingredients, structural formula, physico-chemical quality.

2. Analytical method

(1)Reagent and device (instrument)

(2)Analytical object substance

Reason for selecting analytical object substance, structural formula, and physico-chemical quality

(3)Operating conditions of analyzing devices

(4)Preparation of calibration curve

(5)Analyzing operations

3. Review items on analytical methods

(1)Limit of determination

(2)Recovery test

(3)Standard deviation percentage

(4)Other review items

4. List of Referential Attached Data

•Dissipation curve of test substance in soil (semilogarithmic graphics (concentration is plotted as logarithm)) and DT50

•Table of contents of gas chromatogram, etc. evidencing test achievement and attached diagrams

Attached Sheet 2

(-) Name of laboratory:_____

Specification of Soil Residue Analytical Sample Preparation

(Test period: to)

1. Test Substance
   (1) Generic name/formulation
   _____
   (2) Active Ingredients and Component Percentage Content
   _____
   (3) Lot No. of Test Substance
   _____
2. Name of Agricultural Crop_____Name of Variety _____
   Distinction of Open field and facility_____
3. Sample Preparation Implementing Institution
   _____
   Sample Preparation Ranch Address(Denote the position in a map of one-fifty thousandth)
   _____
4. Name of Staffer in charge of Sample Preparation
   _____
5. Characteristics of soil
   (1) Soil group (soil family group):_____
   (2)Origin of Volcano: General descriptions of volcanic ash/alluvium/diluvium, etc._____
   (3) Mechanical composition and soil texture (classification as per USDA, etc.)
   _____
   (4) Kinds of major clay minerals _____
   (5) Soil pH: (H_2O) _____ (KCl or $CaCl_2$) _____
   (6) Organic carbon content: _____
   (7) Cation ion exchange capacity (CEC):_____
   (8) Others (Phosphoric acid absorption coefficient, the maximum moisture holding capacity, etc.)
   _____
   (9) Decreased water depth (in the case of a paddy field) _____
6. Crops which were planted and agricultural chemicals which were used in past one year (may be written in an Appended sheet). _____
   _____
   _____
   _____
7. Generals of cultivation (seeding time, raising of seedling, transplanting time, kind/quantity/time of fertilizing, harvesting stage, etc.) _____
   _____
   _____
8. Conditions of soil (cultivation, draining, irrigation time, etc.) _____
   _____
   _____
9. Agricultural chemical used other than the test substance (may be written in an appended sheet)
   _____
   _____
   _____
   _____
10. Test area
    (1) Area and No. of strains in one test area _____
    (2) In the case of a facility, area/volume/height
    (3) Layout view of test area (Enter it so as to clarify the entire test area and the conditions of the neighboring agricultural lands, etc.)
    a) Prepare a disposition relationship between a treated area and an untreated area by connecting one dot to another in the following square.
    b) Encircle a dot to indicate one fruiter, and describe the elongation direction of a ramus when necessary.
    c) Enter distances between the test areas and between test area and the untreated area.
    d) Enter the balk direction and orientation of a ranch and the inclination direction of the ranch. Enter water supply ports and drain ports in the paddy fields.

(-) Name of laboratory:_____

11. Treating method

Area / No. of Times	Time of usage / Date	Applied Concentration	Treated quantity		Growth stage at treatment	Treating methods (Entire application, row application, planting hole application, application on water surface, soil injection, etc.)	Applied too	General weather conditions on a day on which a treatment is performed including a treating time, and a weather at treatment (direction of wind, wind velocity)
			Per 10a	Per test area (agricultural chemical dose/diffused dose/area)				
First								
Second								
Third								
Fourth								
Fifth								

(1) Spreader Name_____Concentration or dose _____

(2) Remarks: _____

12. Sampling

Area / No. of times	Sampling time	Sampling		Sampled dose	Weather at sampling	Sending	Remarks	Area / No. of times	Sampling time	Sampling		Sampled dose	Weather at sampling	Sending	Remarks
		Date	No.			Date				Date	No.			Date	
	Untreated area														
	Just before ____Treatment														
	Just after ____Treatment														
	_____days after ____Treatment														
	_____days after ____Treatment														

Sampling method (the details of the applied tool and sampling method) _____

12. Preparation and packaging method after sampled _____

Sample addressee Transportation method of sample

13. Meteorological table (separately attached)

14. Remarks:_____

(-)

Meteorological Table

Observation Point and Sample Preparation Place ____

Air temperature: Average air temperature (by________hours) Precipitation: at_________hour on
Measurement time __________AM

Symbol:○: Agricultural chemical treatment date

△: Sampling date

(Year)

Month	Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
	Temperature																															
	(Temperature in facility)																															
	Precipitation																															
	Treatment / Sampling																															
	Temperature																															
	(Temperature in facility)																															
	Precipitation																															
	Treatment / Sampling																															
	Temperature																															
	(Temperature in facility)																															
	Precipitation																															
	Treatment / Sampling																															
	Temperature																															
	(Temperature in facility)																															
	Precipitation																															
	Treatment / Sampling																															

Record chemical treatment (including aeration, etc.) and sample collection days, using code. Enter code for “temperature inside greenhouse” for greenhouse studies, and as “air temperature” for open field studies. Enter the appropriate code for chemical treatment day, and the appropriate code for sample collection day (same code as on shipping card), in the appropriate columns.

Appended Format 4

Table of Crop Residue Test Results

Date:_____

1.Name of Test Facility: Director's name	(Affiliation)	(Name)	7.Name of sponsor:	
2.Name of Test Substance:		(Type of Formulation)	8.Purpose of usage	
3. Chemical name and content (%) of the active ingredient			9.Applied concentration and quantity and application method of the test substance	
4. Analyte (name of component)			10. Sample preparation place	
5. Name of test crop		(Portion of analysis)	11. Name of analyzing facility	
6. Summary of analytical method				

12. Analytical results

Sample (Place of production, variety, etc.)	Applied concentration/ quantity of test substance	Treatment Date	Sampling Date	Date of sample arrival Date	Number of treatments	Days after treatment	Number of analysis	Analytical value (ppm)			Date of sample analysis *1	Remarks (storage period*2 / storage method, weight / length of sample, etc.)
								(1) (2)	R	average		

*1 Date of sample analysis: Report the start date and end date of analysis (e.g., [date] to [date]).
*2 Storage period: Report the duration from sample arrival to start of analysis (e.g., xx days).