The guidelines related to the study reports

for the registration application of pesticide

Appendix to Director General Notification,
No. 12-Nousan-8147, 24 November, 2000,
Agricultural Production Bureau,
Ministry of Agriculture, Forestry and Fisheries of Japan

Note: This translation is made by Ministry of Health, Labour and Welfare. In the case of any discrepancy between the Japanese original and the English translation, the former will take priority.

(Appendix)

I. Regarding specific details of test results

The specific details of test results in regard to the efficacy, phytotoxicity, toxicity, and persistence of agricultural chemicals that should be presented by an applicant for registration (hereinafter referred to as “the applicant”) of such chemicals (does not include those having microorganisms as active ingredients), based on the stipulations in Article 2, Paragraph 2 (including cases to which Article 15.2, Paragraph 6 apply) and in Article 6.2, Paragraph 1 (including cases to which Article 15.2, Paragraph 6 apply; same below) of the Agricultural Chemicals Regulation Law (No. 82, 1948; hereinafter referred to as “the Law”) are as follows.

(1) Test results in regard to efficacy

Test results in regard to efficacy against the relevant diseases or insect pests (test results in regard to efficacy with respect to the applicable crops, in the case of chemicals used to promote or suppress physiological functions of crops, etc.)

(2) Test results in regard to phytotoxicity

a. Test results in regard to phytotoxicity with respect to applicable crops
b. Test results in regard to phytotoxicity with respect to peripheral crops
c. Test results in regard to phytotoxicity with respect to succeeding crops

(3) Test results in regard to toxicity

a. Acute oral toxicity test results
b. Acute dermal toxicity test results
c. Acute inhalation toxicity test results
d. Skin irritation test results
e. Eye irritation test results
f. Skin sensitization properties test results
g. Acute neurotoxicity test results
h. Acute delayed neurotoxicity test results
i. 90-day repeated dose oral toxicity test results
j. 21-day repeated dermal toxicity test results
k. 90-day repeated inhalation toxicity test results
l. Repeated dose oral neurotoxicity test results
m. 28-day repeated delayed neurotoxicity test results
n. 1-year repeated dose oral toxicity test results
o. Carcinogenicity test results
p. Reproductive toxicity test results
q. Teratogenicity test results

r. Mutagenicity test results

s. Pharmacology test results

t. Test results in regard to prospective fate in animals

u. Test results in regard to prospective fate in plants

v. Test results in regard to prospective fate in soil

w. Test results in regard to prospective fate in water

x. Test results in regard to impact on aquatic animals and plants

y. Test results in regard to impact on beneficial organisms other than aquatic animals and plants

z. Test results in regard to the properties, stability, degradability, etc. of active ingredients

aa. Test results in regard to water polluting properties

(4) Test results in regard to persistence

a. Test results in regard to persistence in crops

b. Test results in regard to persistence in soil

II. Regarding conditions relevant to preparation of test results

The test results cited in Section I must be obtained by implementing the tests cited in the "Test items" column in Appendix Table 1, on the basis of the conditions cited in the "Conditions Necessary for Implementing Test" column in the same table. The test methods are to be those stipulated in the appendix entitled "Guidelines on Preparation of Test Results Submitted When Applying for Registration Of Agricultural Chemicals".

III. Regarding formats of test results

The formats for test results that should be submitted are: Summary of Study Report, containing a summary of the test results cited in Section I, and Test Data Report.

IV. Exceptions as regards submission of test results

If there are reasonable grounds, as regards the type of active ingredients in the relevant agricultural chemical, as listed in Appendix Table 2 or elsewhere, or its formulation, method of use, etc., for regarding submission of some test results as unnecessary, the applicant may submit present said reasons in writing instead of said test results, the stipulations in Section I notwithstanding.

V. Regarding substitutes for test results

(1) If some of the test results that are to be submitted with an application for agricultural chemical registration have already been submitted with another registration application, and if it is deemed possible to use these test results in reference to the agricultural chemical concerned in the relevant registration, the applicant may submit a separate Test Chemical Results Substitution Form (see separate form) in lieu of the relevant test results.

In such cases, if the party submitting the test results to be used is not the applicant, a document must be attached in which the applicant states that he/she has no objection to submission of the test results that are to be used.

(2) If some of the test results that are to be submitted with an application for agricultural chemical registration (limited to Section I (3) a-c and t-w, and (4) a and b) have already been submitted with another registration application, 15 years or more previously, and if it is determined that the agricultural chemical that is the subject of the present application for registration has the same components, physical/chemical properties, toxicity with respect to humans and livestock, and other characteristics, and is a chemical that has been registered 15 or more years previously, the applicant may submit a separate Test Chemical Results Substitution form in lieu of the relevant test results.

VI. Regarding requests for additional test results, etc.

Based on the stipulations in Article 2, Paragraph 3 of the Law, the applicant may be asked to submit test results, etc. in regard to the agricultural chemical applied for, if these are deemed necessary for examination in connection with registration.

VII. Regarding submission of reports on toxicity of agricultural chemicals

Insofar as possible, efforts should be made to submit to the Minister of Agriculture, Forestry, and Fisheries of Japan information that is obtained regarding toxicity of the agricultural chemical concerned in the application, in addition to test results cited in Section I (3), to help in assuring its quality and safety. The same is true after registration of the agricultural chemical.

VIII. Regarding the handling of findings as to the toxicity of agricultural chemicals

The applicant should make efforts to publish findings, obtained in tests regarding the toxicity of agricultural chemicals and as cited in Section I (3) a-s, under the auspices of a scientific association or in a scientific journal, as a rule within 3 years after registration.

IX. This notification applies to test results in regard to efficacy, phytotoxicity, toxicity, and persistence that are submitted on and after February 1, 2001. However, it does not apply to test results regarding agricultural chemicals that are already registered, or to agricultural chemicals that have the same active ingredients as agricultural chemicals that are already registered, or to requests for changes in registration, as stipulated in Article 2, Paragraph 1, Article 15.2, Paragraph 1, and Article 6.2, Paragraph 1 of the Law.

(Separate form)

Test Chemical Results Substitution Form

Year Month Date

To: The Minister of Agriculture, Forestry, and Fisheries of Japan

Address:

Name: (Corporate name, if any and name of representative) Personal seal

I hereby request substitution of test results for registration of agricultural chemicals, as cited below, per Section V of (Notification from the Director-General, Agricultural Production Bureau, (Month) (Date), 2000).

To wit:

1. Type and name of the agricultural chemical (include the registration numbers of agricultural chemicals that were previously registered).

2. Details of the test results to be used as substitutes; the type and name of the agricultural chemicals used in obtaining these test results (include the registration numbers of agricultural chemicals that were previously registered).

(Japan Industrial Standard A4)

Note: Use of a personal seal may be omitted if the applicant (or representative, in the case of a corporate applicant) uses his/her signature.

(Appendix Table 1)

Test Results	Test item	Conditions necessary for implementing tests			
		Type of test substance	Necessary number of CTES /Type of test crops, test animals, etc.	Test institution standards (Note 5) (Note 6)	Implementation method number (see Appendix)
Test results regarding efficacy against relevant diseases and insect pests (test results in regard to efficacy with respect to the applicable crops, in the case of chemicals used to promote or suppress physiological functions of crops, etc.)	Efficacy tests (Note 1)	Formulation (Note 8)	Appears in Appendix Table 1	Public testing and research institution, or an equivalent institution	1-1-1
Test results regarding phytotoxicity with respect to applicable crops	(1) Phytotoxicity tests (Note 2)	Formulation (Note 8)	Appears in Appendix Table 1	Public testing and research institution, or an equivalent institution	1-1-1
	(2) Critical dosage (or concentration) Phytotoxicity tests	Formulation	2 examples of each applicable crop (for crop groups, other conditions stipulated by the head of Agricultural Product Safety Control Division should be met)	No particular stipulations	1-1-2
	(3) Tests of residual odor in tea	Formulation	2 examples	Public testing and research institution, or an equivalent institution	1-1-3
	(4) Tobacco taste tests	Formulation	2 examples (3 examples in cases in which foliage has been directly exposed to the relevant agricultural chemical, or in which the active ingredients of the relevant agricultural chemical have been absorbed via the roots	Public testing and research institution, or an equivalent institution	1-1-4
Test results regarding phytotoxicity on peripheral crops	(1) Tests of phytotoxicity due to drift and scattering	Formulation	Select 1 representative variety from each of the following, based on applicable crops and applicable location: *Solanum*, *Cucurbitaceae*, *Brasicae*, *Legume*, *Gramin*, etc.	No particular stipulations	1-2-1
	(2) Tests of phytotoxicity due to runoff into paddy water	Formulation	Select 1 representative variety from each of the following: rushes, lotus root, arrowhead, etc.	No particular stipulations	1-2-2
	(3) Tests of phytotoxicity due to	Formulation	Select 1 type of crop that is thought to be highly susceptible to the relevant	No particular stipulations	1-2-3

	volatilization		agricultural chemical.		
Test results in regard to phytotoxicity on succeeding crops	Succeeding crop side effect tests	Formulation	From among crops that can be cultivated as crops succeeding those to which the relevant agricultural chemical will be applied, select 1 type that is thought to be highly susceptible to the relevant agricultural chemical.	No particular stipulations	1-3
Test results in regard to acute oral toxicity	Acute oral toxicity tests	Technical grade active ingredient (TGAI) and formulation	1 type of test animal for each test substance (usually rats)	Test facilities conforming to GLP standards for agricultural chemicals	2-1-1
Test results in regard to acute dermal toxicity	Acute dermal toxicity tests	TGAI and formulation	1 type of test animal for each test substance (usually rats, rabbits, or guinea pigs)	Test facilities conforming to GLP standards for agricultural chemicals	2-1-2
Test results in regard to acute inhalation toxicity	Acute inhalation toxicity tests	TGAI and formulation	1 type of test animal for each test substance (usually rats)	Test facilities conforming to GLP standards for agricultural chemicals	2-1-3
Test results regarding acute skin irritation	Skin irritation tests	Formulation (or TGAI, if formulation is difficult to use)	1 type of test animal (usually rabbits)	Test facilities conforming to GLP standards for agricultural chemicals	2-1-4
Test results regarding eye irritation	Eye irritation tests	Formulation (or TGAI, if formulation is difficult to use)	1 type of test animal (usually rabbits)	Test facilities conforming to GLP standards for agricultural chemicals	2-1-5
Test results regarding skin sensitization	Skin sensitization tests	TGAI and formulation	1 type of test animal for each test substance (usually guinea pigs)	Test facilities conforming to GLP standards for agricultural chemicals	2-1-6
Test results regarding acute neurotoxicity	Acute neurotoxicity tests	TGAI	1 type of test animal (usually rats)	Test facilities conforming to GLP standards for agricultural chemicals	2-1-7
Test results regarding acute delayed neurotoxicity	Acute delayed neurotoxicity tests	TGAI	1 type of test animal (usually chickens)	Test facilities conforming to GLP standards for agricultural chemicals	2-1-8
Test results regarding 90-day repeated dose oral toxicity	90-day repeated dose oral toxicity tests	TGAI	2 types of test animals (usually rats and dogs) (However, it is acceptable to use only 1 type of test animal if the test substance is considered safe on the grounds that there is no danger of long-term exposure of humans to its components, due to its formulation and methods of use.)	Test facilities conforming to GLP standards for agricultural chemicals	2-1-9

Test results regarding 21-day repeated dermal toxicity	21-day repeated dermal toxicity tests	TGAI	1 type of test animal (usually rats, rabbits, or guinea pigs)	Test facilities conforming to GLP standards for agricultural chemicals	2-1-10
Test results regarding 90-day repeated inhalation toxicity	90-day repeated inhalation toxicity tests	TGAI	1 type of test animal (usually rats)	Test facilities conforming to GLP standards for agricultural chemicals	2-1-11
Test results regarding repeated dose oral neurotoxicity	Repeated dose oral neurotoxicity tests	TGAI	1 type of test animal (usually rats)	Test facilities conforming to GLP standards for agricultural chemicals	2-1-12
Test results regarding 28-day repeated administration delayed neurotoxicity	28-day repeated administration delayed neurotoxicity tests	TGAI	1 type of test animal (usually chickens)	Test facilities conforming to GLP standards for agricultural chemicals	2-1-13
Test results regarding 1-year repeated dose oral toxicity	1-year repeated dose oral toxicity tests (Combined repeated dose oral toxicity/ carcinogenicity tests) (Note 3)	TGAI	2 types (usually rats and dogs) 1 type may be concurrently used in carcinogenicity tests.	Test facilities conforming to GLP standards for agricultural chemicals	2-1-14 (2-1-16)
Test results regarding carcinogenicity	Carcinogenicity tests (Combined repeated dose oral toxicity/ carcinogenicity tests) (Note 4)	TGAI	2 types of test animal (usually rats and dogs) 1 type may be concurrently used in 1-year repeated dose oral toxicity tests.	Test facilities conforming to GLP standards for agricultural chemicals	2-1-15 (2-1-16)
Test results regarding reproductive toxicity	Reproductive toxicity tests	TGAI	1 type of test animal (usually rats)	Test facilities conforming to GLP standards for agricultural chemicals	2-1-17
Test results regarding teratogenicity	Teratogenicity tests	TGAI	2 types of test animals (usually rats and dogs) When conducting reproductive toxicity tests, 1 type should be of the same species and strain as the test animals used in those tests.	Test facilities conforming to GLP standards for agricultural chemicals	2-1-18
Test results regarding mutagenicity	(1) Reverse mutation tests	TGAI	1 example (implement using bacteria)	Test facilities conforming to GLP standards for agricultural chemicals	2-1-19-1
	(2) Chromosomal aberration tests	TGAI	1 example (implement using cultured mammalian cells)		2-1-19-2
	(3) Micronucleus tests	TGAI	1 example (implement using cultured mammalian cells)		2-1-19-3

Test results regarding pharmacology	Pharmacology tests	TGAI	1 example (implement using species of animals appropriate for each test item)	Test facilities conforming to GLP standards for agricultural chemicals	2-2-1
Test results regarding prospective fate in animals	Tests of prospective fate in animals	Active ingredients, etc. that are either labeled or unlabeled with radioactive istopes	1 type of test animal (ordinarily rats)	Test facilities conforming to GLP standards for agricultural chemicals	2-3-1
Test results regarding prospective fate in plants	Tests of prospective fate in plants	Active ingredients, etc. that are either labeled or unlabeled with radioactive istopes	Shown in Appendix Table 1	Test facilities conforming to GLP standards for agricultural chemicals	2-4-1
Test results regarding prospective fate in soil	(1) Tests of prospective fate in flooded aerobic soil	Active ingredients, etc. that are either labeled or unlabeled with radioactive istopes	1 example	Test facilities conforming to GLP standards for agricultural chemicals	2-5-1
	(2) Tests of prospective fate in aerobic soil	Active ingredients, etc. that are either labeled or unlabeled with radioactive istopes; however, tests may also be implemented in regard to major metabolites that are detected by means of these tests, when deemed necessary on the basis of tests of prospective fate in aerobic soil.	1 example	Test facilities conforming to GLP standards for agricultural chemicals	2-5-2
	(3) Tests of prospective fate in anaerobic soil	Active ingredients, etc. that are either labeled or unlabeled with radioactive istopes; however, tests may also be implemented in regard to major metabolites that are detected by means of these tests, when deemed necessary on the basis of tests of prospective fate in aerobic soil.	1 example	Test facilities conforming to GLP standards for agricultural chemicals	2-5-3

Test results regarding prospective fate in water	(1) Tests of hydrolytic fate	Active ingredients, etc. that are either labeled or unlabeled with radioactive istopes	1 example	Test facilities conforming to GLP standards for agricultural chemicals	2-6-1
	(2) Tests of photolytic fate in water	Active ingredients, etc. that are either labeled or unlabeled with radioactive istopes	1 example	Test facilities conforming to GLP standards for agricultural chemicals	2-6-2
Test results regarding impact on aquatic animals and plants	(1) Fish acute toxicity tests	TGAI or formulation	1 example for each test substance (usually conducted with common carp or killifish)	Test facilities conforming to GLP standards for agricultural chemicals	2-7-1
	(2) *Daphnia spp* acute immobilization tests	TGAI or formulation	1 example for each test substance	Test facilities conforming to GLP standards for agricultural chemicals	2-7-2-1
	(3) *Daphnia spp* reproductive tests	TGAI	1 example	Test facilities conforming to GLP standards for agricultural chemicals	2-7-2-2
	(4) Algae growth inhibition tests	TGAI or formulation	1 example for each test substance	Test facilities conforming to GLP standards for agricultural chemicals	2-7-3
Test results regarding impact on beneficial organisms other than aquatic animals and plants	(1) Bee impact tests	TGAI or formulation	1 example	No particular stipulations	2-8-1
	(2) Silkworm impact tests	TGAI or formulation	1 example	No particular stipulations	2-8-2
	(3) Tests of impact on natural enemy insects, etc.	TGAI or formulation	Choose 2 classes and 3 species from among Diptera, Hymenoptera, Hemiptera, Coleoptera, Neuroptera, Acarina, and Arachnida	No particular stipulations	2-8-3
	(4) Avian impact tests				
	(i) Avian forced oral administration tests	TGAI	1 example	No particular stipulations	2-8-4-1
	(ii) Avian dietary toxicity test	TGAI	1 example	No particular stipulations	2-8-4-2
Test results regarding the properties, stability, degradability, etc. of active ingredients	Color tests, tests of substance form, odor tests, spectrum tests, melting point tests, boiling point tests, vapor pressure tests, tests of solubility in water,	Active ingredients in their pure state (Note 7) (or TGAI, if it is too difficult to implement tests with active ingredients in their pure state; if active	1 example for each test substance	Test facilities conforming to GLP standards for agricultural chemicals (except for color tests, tests of substance form, and odor tests)	2-9-1~16

	tests of solubility in organic solvent, soil absorbency tests, octanol/water partition coefficient tests, density tests, hydrolyzability tests, dissociation constant tests, tests of stability with respect to heat, test of photolysis in water	ingredients are composed of multiple chemical compounds, separate them if possible, and treat them as separate substances)			
Test results regarding water polluting properties	Tests of water polluting properties	Formulation	2 examples	Public testing and research institution, or an equivalent institution	2-10-1

Test results regarding persistence in crops	(1) Tests of persistence in crops	Formulation (Note 8)	2 examples for each crop (For crop groups, other conditions stipulated by the head of Agricultural Product Safety Control Division should be met) that is to receive application. Implement tests according to the following standards. (i) Implement tests in different prefectures that are major areas of cultivation for the relevant crop. (ii) If it is difficult to comply with the standard set in (i) above, because the relevant crop is only produced in the specified areas, conduct the tests in multiple locations within a single prefecture, or during multiple years in the same location.	Public testing and research institution, or an equivalent institution. Samples should be formulated according to the following standards. (1) To be conducted in different prefectures that are major areas of cultivation for the relevant crop. (2) For products of low production volume, or those produced in cultivation areas in a single prefecture, the study should be conducted at more than one area in the prefecture or at a fixed area for more than one year. Sample analysis facilities should comply with the following standards. (1) Analysis should be conducted twice in a row. At least one analysis should be conducted in an official testing and research institution, or equivalent institution. (2) For crops of low production volume, a single analysis can be conducted in an official testing and research institution, or equivalent institution.	3-1-1
	(2) Tests of translocation to milk	TGAI or formulation	1 example (Conduct tests with cows during their lactation period.)	No particular stipulations	3-1-2
Test results regarding persistence in soil	(1) Soil persistence tests				
	(i) Tests in containers	TGAI or active ingredients in their pure state	2 examples	No particular stipulations	3-2-1-1

	(ii) Field tests	Formulation	2 examples	Public testing and research institution, or an equivalent institution. There are no particular stipulations as regards conditions in the institutions.	3-2-1-2
	(2) Tests of persistence in succeeding crops	Formulation	(1) For tests with agricultural chemicals for use in paddies, select 1 type of crop from among root vegetables, and 1 type from among grains, beans, or other crops. (2) For tests with agricultural chemicals for use in dry fields, select 1 type of crop from among root vegetables, and 1 type from among types of plant that could conceivably be grown therein as succeeding crops.	No particular stipulations	3-2-2

Note 1: To be implemented concurrently with phytotoxicity tests.

Note 2: To be implemented concurrently with efficacy tests.

Note 3: May be implemented concurrently with carcinogenicity tests.

Note 4: May be implemented concurrently with 1-year repeated dose oral toxicity tests.

Note 5: “Public testing and research institution” refers to institutions that meet administrative requirements for implementation of tests therein by national and local authorities.

Note 6: An institution that is “equivalent” to a public testing and research institution is one that meets administrative requirements for implementation of tests therein by parties that are non-profit organizations, or that clearly has no conflict of interest as regards the applicant, and that is capable of properly implementing the relevant tests.

Note 7: “Pure state” means, in principle, at least 98% pure.

Note 8: This is a combination of spreader and the agricultural chemical to be applied.

(Additional Table 1)

Test Items	Necessary number of CTES
Efficacy tests and phytotoxicity tests (and concurrent efficacy / phytotoxicity tests)	Tests on crops (for crop groups, products belonging to the group should be included (As a rule, chemical herbicides and herbal growth regulators are excluded. The same applies to the following part of this table)), insect pests, weeds or combination of these to which the substance is to be applied, should be conducted for at least 2 years. The tests conducted each year should, in principle, be conducted in at least 3 locations in different prefectures. However, in the following cases, the number of tests implemented may be as suggested below. (1) If the agricultural chemical relevant to the application that is to be applied to crops, diseases, insect pests, or weeds, or a combination of these, is the same as one that is already registered, and if it satisfies any of the following conditions, tests on each of the crops, diseases, insect pests, or weeds, or combination of these to which the substance will be applied should, in principle, be conducted in institutions in at least 3 locations in different prefectures. (i.) The substance has the same active ingredients as the registered agricultural chemical, but in a different formulation. (ii) The substance has the same active ingredients and formulation as the registered agricultural chemical, but a lesser amount of the active ingredient is to be released than would be the case with the registered agricultural chemical. (iii.) The substance is a mixture including active ingredients from multiple registered agricultural chemicals, but the content of each active ingredient in the relevant agricultural chemical is different from the content of the respective active ingredients in the registered agricultural chemical. (iv.) The substance is a registered agricultural chemical, but either the concentration or the dosage (the amount of active ingredients released) have been reduced. (v.) The substance is a registered agricultural chemical, but the method of use has been changed. (2) If the agricultural chemical relevant to the application, to be applied to crops, diseases, insect pests, or weeds, or a combination of these, is the same as one that is already registered, and if it satisfies any of the following conditions, tests on each of the crops, diseases, insect pests, or weeds, or combination of these to which the substance will be applied should, in principle, be conducted in institutions in at least 2 locations in different prefectures. (i.) The substance has the same active ingredients and formulation as the registered agricultural chemical; the amount of active ingredient released is either the same as or greater than would be the case with the registered agricultural chemical. (ii.) The substance is a mixture including active ingredients from multiple registered agricultural chemicals, and the content of each active ingredient in the relevant agricultural chemical is the same as the content of the respective active ingredients in the registered agricultural chemical. (iii.) The substance is a registered agricultural chemical, but either the concentration or the dosage (the amount of active ingredients released) have been increased. (3) If the agricultural chemical relevant to the application satisfies any of the following conditions, tests on each of the

	crops, diseases, insect pests, or weeds, or combination of these to which the substance will be applied should, in principle, be conducted in institutions in at least 2 locations in different prefectures. (i.) The substance is a registered agricultural chemical, but it is used for a secondary purpose, against diseases, harmful insects, and weeds, in addition to those for which it was originally intended. (ii.) The substance is a registered agricultural chemical, and is to be applied to additional test crops that are similar to those for which it was originally intended, as a measure against diseases, insect pests, weeds, etc. (iii.) The substance is to be applied to crops that are only grown in a limited region, or that are produced in low volumes. (iv.) The substance is to be used against diseases, insect pests, weeds, etc. that only occur in limited areas. (v.) The substance is a registered agricultural chemical, and there is an urgent need to widen the range of diseases, insect pests and weeds to which it may be applied, in order to provide plant protection. (vi.) The application is for a spreader. (4) If the agricultural chemical relevant to the application satisfies any of the following conditions, tests on each of the crops, diseases, insect pests, or weeds, or combination of these to which the substance will be applied should, in principle, be conducted in institutions in at least 3 locations in different prefectures. (i.) The substance is a mixture including new active ingredients as well as the active ingredients of a registered agricultural chemical, and it is to be applied to crops, diseases, insect pests, weeds or a combination of these that include crops, diseases, insect pests, weeds or a combination of these that are the same as those to which the registered agricultural chemical is applied; tests are to be conducted only in regard to the active ingredients of the registered chemical, as applied to crops, diseases, insect pests, weeds or a combination of these. (ii.) The substance is a registered agricultural chemical, and is to be used on additional crops that share in common with crops for which the registered agricultural chemical were originally intended against diseases and insects pests that are difficult to control. (iii.) The substance is a registered agricultural chemical, and is to be used on crops to which the registered chemical is applied as a measure against diseases and insect pests, in circumstances in which no crops are present, or the chemical will not come into contact with crops. (5) Tests should be conducted in at least 3 locations for each crop, as well as each disease or insect pest concerned in the registration application, with regard to agricultural chemicals that are used in warehouses, silos, etc.
Tests regarding prospective fate in plants	Conduct tests after selecting at least 1 type of crop from among the crops cited in the right column of Additional Table 2 for each classification of crops relevant to the registration application. However, if there are at least 3 types of crop classification relevant to the registration application, and a large difference is observed in the metabolism of crops involved in each classification, 3 plant classes may be used in testing. If the crops concerned in the registration application are limited to 1 class, and the test plants are different from the crops concerned in the application, used 2 or more types of test plants.

	If rice is included as one of the plants to which the substance will be applied, the test crops must include paddy rice. For target crop containing a genetically modified product, the genetically modified product should be examined in addition to those selected by the above-defined method.

(Additional Table 2)

○ Classification of plants as subjects for tests regarding prospective fate in plants

Plant classes	Main crops
Rice	Paddy rice
Grains and sugar cane	Wheat, barley, rye, corn (maize), buckwheat, sugar cane
Fruit (not including citrus fruit or gourds)	Peaches, loquats, kiwis, apples, pears, persimmons, nectarines, apricots, cherries, Japanese apricots, strawberries, grapes, gingko nuts, chestnuts, walnuts
Citrus fruit	Wenzhou mandarin orange, large citrus fruit, small citrus fruit
Fruit vegetables (including gourds)	Sweet peppers, okra, chili peppers, squashes, cucumbers, tomatoes, eggplants, watermelons, melons
Plants with edible leaves or flowers	Cabbage, Chinese cabbage, daikon leaves, broccoli, young soybeans in the pod, field peas, string beans, onions, carrot, scallions, hops
Plants with edible roots or stalks	Daikon roots, carrots, ginger, potatoes, sweet potatoes, taro, sugar beet
Beans and oil-producing vegetables	Soybeans, adzuki beans, peas, fava beans, rape seed, sesame seeds, safflower
Mushrooms	*Shiitake*, *enokitake*
Tea plant	Leaves of tea, fruits (excluding citrus and squash) and citrus plant groups

(Appendix Table 2)

In Section IV, the phrase “as listed in Appendix Table 2” refers to circumstances described in the right column, for each of the types of test results in the left column, of the following table.

Type of test results	Circumstances in which test results need not be submitted
Test results regarding phytotoxicity in regard to relevant crops	
(1) Tests of residual odor in tea	When tea is not among the applicable crops
(2) Tobacco taste tests	When tobacco is not among the applicable crops
(3) Tests of critical dosage (or concentration) for phytotoxicity	When it is determined, in terms of the method by which the relevant agricultural chemical is to be used, that there is no danger that the target crops will be exposed to the relevant chemical beyond the ranges (dosage, concentration) in which it is to be applied
Test results regarding phytotoxicity in regard to peripheral crops	
(1) Test results regarding phytotoxicity due to drift and scattering	When it is determined, in terms of the type, formulation, method of use, etc. of the relevant agricultural chemical, that there is no danger of it affecting (being phytotoxic to) peripheral crops through drifting and scattering
(2) Test results regarding phytotoxicity due to runoff into paddy water	Circumstances that fall into either of the following categories: (i.) When the chemical is not to be used in paddies (ii.) When it is determined, in terms of the method by which the relevant agricultural chemical is to be used, that there is no danger of it affecting (having phytotoxicity on) peripheral crops through runoff into water systems such as rivers, via paddy water
(3) Test results regarding phytotoxicity due to volatilization	When it is determined, in terms of the properties of the active ingredients of the relevant agricultural chemical, or its formulation, method of use, etc., that there is no danger of it affecting (having phytotoxicity on) peripheral crops through volatilization
Test results regarding phytotoxicity in regard to succeeding crops	When it is determined, in terms of the method by which the relevant agricultural chemical is to be used, and in terms of the degree of persistence in soil, that there is no danger of it affecting (having phytotoxicity on) crops that are to be cultivated after the crops to which said chemical is to be applied
Acute dermal toxicity test results	When it is determined that the relevant agricultural chemical is corrosive (as are, for example, strong acids (in general, those of pH $\leq$2) and strong alkalines (in general, those of pH $\geq$11.5)

Acute inhalation toxicity test results	When it is determined that there is no danger that users will be exposed to the relevant agricultural chemical through inhalation, in terms of its formulation and method of use, etc. in connection with its implementation
Skin irritation test results	When it is determined that the relevant agricultural chemical is corrosive (as are, for example, strong acids (in general, those of pH $\leq$ 2) and strong alkalines (in general, those of pH $\geq$ 11.5)
Eye irritation test results	Circumstances that fall into either of the following categories: (i) When it is determined that the relevant agricultural chemical is corrosive (as are, for example, strong acids (in general, those of pH $\leq$ 2) and strong alkalines (in general, those of pH $\geq$ 11.5) (ii.) When it seems unlikely that the chemical is corrosive, based on skin irritation tests
Acute neurotoxicity test results	When it is determined, based on the results of acute toxicity tests, that there is no danger of neurotoxicity
Acute delayed neurotoxicity test results	Circumstances that fall into either of the following categories: (i.) When it is determined, based on the results of acute toxicity tests, that there is no danger of delayed neurotoxicity (ii.) When it is determined that there is no danger of delayed neurotoxicity, in terms of correlations with the chemical structures of chemicals that are known to have delayed neurotoxic effects
90-day repeated dose oral toxicity test results	Circumstances that fall into either of the following categories: (i.) When the relevant agricultural chemical is determined to be safe, in terms of the method by which it is to be used, because the amount of exposure to substances that are ingredients of the relevant agricultural chemical (including other substances that are produced through chemical changes in these substances) is very slight, etc. when it is employed (ii.) When the relevant agricultural chemical is determined to be safe, in terms of the type of components it has, etc., because they are of very low toxicity, etc.
21-day repeated dermal toxicity test results	Circumstances that fall into either of the following categories: (i.) When it is determined that there is no danger of prolonged skin exposure to the relevant agriculture chemical on the part of persons who are applying it (ii.) When it is determined, on the basis of acute dermal toxicity test results, that there is no danger that the chemical has a high dermal toxicity
90-day repeated inhalation toxicity test results	Circumstances that fall into either of the following categories: (i.) When it is determined that there is no danger of prolonged inhalation exposure to the relevant agriculture chemical on the part of persons who are applying it (ii.) When it is determined, on the basis of test results in regard to acute inhalation toxicity, that there is no danger that the chemical has a high inhalation toxicity

Repeated dose oral neurotoxicity test results	When it is determined, based on the results of 90-day repeated dose oral toxicity tests, that there is no danger of neurotoxicity
28-day repeated administration delayed neurotoxicity test results	When it is determined, based on the results of acute delayed neurotoxicity tests, that there is no danger of delayed neurotoxicity
1-year repeated dose oral toxicity tests	Circumstances that fall into either of the following categories: (i.) When it is determined, in terms of the relevant agricultural chemical's formulation and the method by which it is to be used, that it is safe, because there is no danger of long-term ingestion of the relevant agricultural chemical by humans, or because only a extremely small amount of the chemical's components would be ingested by humans (ii.) When it is determined, in terms of the type of components in the relevant agricultural chemical, that it is safe, because these have an extremely low toxicity
Carcinogenicity test results	Circumstances that fall into either of the following categories: (i.) When it is determined, in terms of the relevant agricultural chemical's formulation and the method by which it is to be used, that it is safe, because there is no danger of long-term ingestion of the relevant agricultural chemical by humans, or because only a extremely small amount of the chemical's components would be ingested by humans, and when no mutagenicity has been clearly confirmed (ii.) When it is determined, in terms of the type of components in the relevant agricultural chemical, that it is safe, because these have an extremely low toxicity
Reproductive toxicity test results	Circumstances that fall into either of the following categories: (i.) When it is determined, in terms of the relevant agricultural chemical's formulation and the method by which it is to be used, that it is safe, because there is no danger of long-term ingestion of the relevant agricultural chemical by humans, or because only a extremely small amount of the chemical's components would be ingested by humans (ii.) When it is determined, in terms of the type of components in the relevant agricultural chemical, that it is safe, because these have an extremely low toxicity
Teratogenicity test results	Circumstances that fall into either of the following categories: (i.) When the relevant agricultural chemical is determined to be safe, in terms of the method by which it is to be used, because the amount of exposure to or ingestion of substances that are ingredients of the relevant agricultural chemical is very slight, etc. when it is employed (ii.) When the relevant agricultural chemical is determined to be safe, in terms of the type of components it has, etc., because they are of very low toxicity, etc.
Test results regarding mutagenicity	The same as in the case of teratogenicity test results

Test results regarding pharmacology	The same as in the case of teratogenicity test results
Test results regarding prospective fate in animals	The same as in the case of teratogenicity test results
Test results regarding prospective fate in plants	Circumstances that fall into any of the following categories: (i.) When the chemical is for use on crops other than those that are to be used for food (including industrial crops and crops to be used for animal feed) (ii.) When it is determined, in terms of the relevant agricultural chemical's formulation and the method by which it is to be used, that it is safe, because there is no danger of long-term ingestion of the relevant agricultural chemical by humans, or because only a extremely small amount of the chemical's components would be ingested by humans (iii.) When it is determined, in terms of the type of components in the relevant agricultural chemical, that it is safe, because these have an extremely low toxicity (iv.) In case an approved agricultural chemical that has already been applied to other edible crops is newly applied to an agricultural product of low production volume.
Test results regarding prospective fate in soil	Circumstances that fall into either of the following categories, or in any of the circumstances below in the right column corresponding to test results (1)-(3) in the left column: (i.) When it is determined, in terms of the relevant agricultural chemical's formulation and the method by which it is to be used, that there is no danger that its components will be mingled with farmland soil (ii.) When it is determined, in terms of the type of components in the relevant agricultural chemical, that it is safe, because these have an extremely low toxicity
(1) Test results regarding prospective fate in flooded aerobic soil	When the substance will not be used in paddies
(2) Test results regarding prospective fate in aerobic soil	When the substance will only be used in paddies; however, this does not include cases in which the tests are thought necessary, based on rate at which the components of the relevant agricultural chemical are dissipated in flooded aerobic soil
(3) Test results regarding prospective fate in anaerobic soil	Circumstances that fall into any of the following categories: (i.) When the substance will only be used in paddies (ii.) When it is found, based on the results of tests of prospective fate in aerobic soil, that the components of the relevant agricultural chemical in aerobic soil are eliminated quickly (iii.) When the relevant agricultural chemical is determined to be safe, as regards its physical/chemical properties, because of its low mobility in soil, etc.

Tests of prospective fate in water	
(1) Test results regarding hydrolytic fate	Circumstances that fall into either of the following categories: (i.) When it is determined, in terms of the relevant agricultural chemical's formulation, the method by which it is to be used, etc. that there is no danger of runoff of its components into water systems such as rivers (ii.) When the relevant agricultural chemical is determined to be safe, in terms of the type of components it has, etc., because they are of very low toxicity, etc.
(2) Test results regarding photolytic fate in water	The same as in the case of test results regarding hydrolytic fate
Test results regarding impact on aquatic animals and plants	
(1) Fish acute toxicity test results	Circumstances that fall into either of the following categories: (i.) When it is determined that there will be no phytotoxicity, based on the extremely low toxicity of the type of components in the relevant agricultural chemical, when implemented as TGAI (ii.) When it is determined, in terms of the relevant agricultural chemical's formulation, the method by which it is to be used, etc. that there is no danger of runoff of its components into water systems such as rivers, when implemented as formulation
(2) *Daphnia spp* acute immobilization test results	The same as in the case of test results regarding fish acute toxicity test results
(3) *Daphnia spp* reproductive test results	When there is no danger of impact of impact on crustaceans, in terms of the type of components in the relevant agricultural chemical, because of their extremely low toxicity
(4) Algae growth inhibition test results	Circumstances that fall into either of the following categories: (i.) When it is determined that there will be no phytotoxicity, based on the extremely low toxicity of the type of components in the relevant agricultural chemical, when implemented as TGAI (ii.) When it is determined, in terms of the relevant agricultural chemical's formulation, the method by which it is to be used, etc. that there is no danger of runoff of its components into water systems such as rivers, when implemented as formulation
Test results regarding impact on beneficial organisms other than aquatic animals and plants	When it is determined that there will be no phytotoxicity, based on the extremely low toxicity of the type of components in the relevant agricultural chemical, or in any of the circumstances below in the right column corresponding to test results (1)-(4) in the left column:
(1) Bee impact test results	When it is determined, in terms of the relevant agricultural chemical's formulation, the method by which it is to be used, etc. that there is no danger that bees will be exposed to it

(2) Silkworm impact test results	When it is determined, in terms of the relevant agricultural chemical's formulation, the method by which it is to be used, etc. that there is no danger that silkworms will be exposed to it through ingesting mulberry leaves, etc.
(3) Test results regarding impact on natural enemy insects, etc.	When it is determined, in terms of the relevant agricultural chemical's formulation, the method by which it is to be used, etc. that there is no danger that natural enemy insects will be exposed to it
(4) Avian impact test results Avian forced oral administration test results Avian dietary toxicity test results	(i.) When it is determined, in terms of the relevant agricultural chemical's formulation, the method by which it is to be used, etc. that there is no danger that birds will be exposed to it (ii.) As regards avian dietary toxicity test results, when it is not confirmed that the substance is highly toxic, based on avian forced oral administration test results
Test results regarding the properties, stability, degradability, etc. of components	Circumstances that fall into any of the following categories: (i.) When the relevant agricultural chemical is determined to be safe, in terms of the type of components it has, etc., because they are of very low toxicity, etc. (ii.) As regards soil absorbency, hydrolysis, and photolysis test results, when it is determined, based on the method by which the relevant agricultural chemical is to be used, etc. that there is no danger that its components etc. will be mingled with farmland soil, or with runoff into water systems such as rivers, when they are used for the purpose intended (iii.) As regards test results for hydrolysis and photolysis, prospective fate in water, when the results which would be the test objectives have already been obtained as results of tests of prospective fate in water
Test results regarding water polluting properties	Circumstances that fall into either of the following categories: (i.) When the substance is not to be used in paddies (ii.) When it is determined that there will be no phytotoxicity, based on the extremely low toxicity of the type of components in the relevant agricultural chemical, when it is used in paddies
Test results regarding persistence in crops	
Test results regarding persistence in crops	Circumstances that fall into either of the following categories: 1. Circumstances that fall into any of the following sub-categories: (i.) When the chemical is for use on crops other than those that are to be used for food (including industrial crops and crops to be used for animal feed) (ii.) When it is determined, in terms of the relevant agricultural chemical's formulation and the method by which it is to be used, that it is safe, because there is no danger of long-term ingestion of the relevant agricultural chemical by humans, or because only a extremely small amount of the chemical's components would be ingested by humans

	(iii.) When it is determined, in terms of the type of components in the relevant agricultural chemical, that it is safe, because these have an extremely low toxicity 2. Circumstances that, regardless of circumstances such as those described in item 1 above, fall into any of the following sub-categories with regard to spreader: (i.) When it is for use on crops other than those that are to be used for food (ii.) Cases in which it is determined that there is no danger that the spreader will affect the persistence of the relevant agricultural chemical in the crops to which it is applied, and it is determined that it is safe, because there is no danger of long-term ingestion of the relevant agricultural chemical by humans, or because only a extremely small amount of the chemical's components would be ingested by humans (iii.) Cases in which it is determined that there is no danger that the spreader will affect the persistence of the relevant agricultural chemical in the crops to which it is applied, and it is determined that it is safe, because of the extremely low toxicity of the type of components in the spreader
Test results regarding translocation to milk	Circumstances that fall into either of the following categories: (i.) When the substance is for use on crops other than those that are to be used for domestic animal feed (ii.) Cases in which it is determined that the substance is safe, since, even though it is for use on crops that are to be used for domestic animal feed, the components, etc. of the relevant agricultural chemical will not persist in crops on which it is used, or if it does persist, only in extremely small traces
Test results regarding persistence in soil	
Soil persistence test results	Circumstances that fall into either of the following categories: (i.) When it is determined, in terms of the relevant agricultural chemical's formulation and the method by which it is to be used, that there is no danger that its components will be mingled with farmland soil (ii.) When it is determined, in terms of the type of components in the relevant agricultural chemical, that it is safe, because these have an extremely low toxicity
Test results regarding persistence in succeeding crops	When it is determined that the relevant agricultural chemical is safe, because there is no danger that crops cultivated after the crops to which it is applied will be polluted by its components

Guidelines for Preparation of Study Results Submitted When Applying for Registration of Agricultural Chemicals

Annex to Director General Notification,
No. 12-Nousan-8147, 24 November, 2000,
Agricultural Production Bureau,
Ministry of Agriculture, Forestry and Fisheries of Japan

Note: This translation is made by Ministry of Health, Labour and Welfare. In the case of any discrepancy between the Japanese original and the English translation, the former will take priority.

(Annex)

Guidelines for Preparation of Study Results Submitted When Applying for Registration of Agricultural Chemicals

Basic Items

1. Basic approach

(1) These guidelines are to be used as a yardstick when preparing study results on efficacy, phytotoxicity, toxicity, and residue that are relevant to applications for registration of agricultural chemicals.

(2) Persons conducting studies are not required to strictly follow these guidelines. Moreover, this guide need not preclude persons conducting studies from making changes in study methods for the purpose of more accurately achieving study objectives in accordance with the properties of the substances to be studied.

2. Regarding substances studied

(1) When technical grade active ingredients (hereinafter referred to as "TGAI") are used as test substances, they must be equivalent to raw materials in agricultural chemical samples.

(2) When formulations are used as test substances, they must be equivalent to agricultural chemical samples.

(3) Test substances from the same lot must be used during the study period. If test substance from another lot must be used, it must be sufficiently similar in composition to the previous lot. Note that number of the lot used must be clearly indicated in the study results.

(4) The composition of each TGAI used in acute toxicity, repeated dose toxicity, carcinogenicity, reproductive toxicity, teratogenicity, and mutagenicity studies must be clearly indicated in the study results.

3. Regarding test organisms

In order to accurately conduct safety evaluations of agricultural chemicals, it is desirable to use test organisms of the same species and strain for all study items.

4. Regarding handling of experimental test organisms

When conducting experiments using animals, sufficient attention must be paid to feeding, experimental procedures, methods of treatment, etc. in accordance with the Law Regarding Prevention of Cruelty to Animals, and Their Care (1973, Law No. 105), Standards for Feeding and Care of Experimental Animals (March 27, 1980, Prime Minister's Office Announcement No. 6), international regulations and trends regarding prevention of cruelty to animals, etc. in order to protect animals from unnecessary suffering.

<Efficacy Studies>

Efficacy study for target pests

Efficacy and phytotoxicity studies (1-1-1)

1. Objective

The objective of these studies is to obtain scientific information concerning the effectiveness of agricultural chemicals (hereinafter referred to as "efficacy") in controlling disease, insect pests and in weeds, and concerning crop phytotoxicity.

2. Test crops

Representative varieties of the target crops.

3. Study methods

(1) Studies are to be carried out in fields (or in greenhouses, etc. when applicable). In order to achieve the study objectives, establish chemically treated and untreated sections of sufficient area, as well as, in principle, a section treated with a control chemical.

Conduct chemical treatment in the chemically treated section according to the methods of use and the dosage (concentration) relevant to the registration application.

(2) As regards chemical treatments, select approproate time period for evaluation of efficacy and phytotoxicity under appropriate conditions as regards occurrence of disease, insect pests, and weeds, as well as the growth stage of crops.

(3) In conducting studies, select appropriate methods based on consideration of the respective properties of the test chemicals, the target diseases, insect pests, and weeds, as well as the target crops.

4. Report items

(1) Efficacy in the chemically treated section as compared to the untreated section and the control section

(2) Whether or not there is phytotoxicity; if so, their nature, degree (measurements of plant height, etc.), extent of recovery, etc.

(3) Other items

(i) Information regarding crops (growth details, growth stage at the time of treatment, etc.)

(ii) Conditions under which the target diseases, insect pests, and weeds occur

(iii) Weather conditions during the study period (temperature, rainfall, etc.)

<Phytotoxicity Studies>

Studies of phytotoxicity on target crops (1-1-1~4)

Efficacy and phytotoxicity studies (1-1-1)

Same as above.

Studies of limit dosage (or concentration) for phytotoxicity (1-1-2)

1. Objective

The objective of these studies is to clarify the maximum dosage and maximum concentration at which phytotoxicity do not occur, and to obtain scientific information concerning target crop phytotoxicity.

2. Test crops

Use a representative variety of crops that are relevant to the registration application, and, in principle, crops that are healthy and at the stage of growth of maximum susceptibility.

3. Study methods

(1) The studies are conducted with the objective of clarifying the maximum dosage or maximum concentration at which phytotoxicity does not occur; however, this dose not need prevent the studies from being conducted double the maximum dosage that would actually be used. In such cases, conduct the study with a dosage (or concentration) limited to twice the maximum dosage that would actually be used in the method of use relevant to the application for registration. Establish a section treated with this maximum dosage, and an untreated section.

(2) Conduct the study according to methods by which sufficient information will be obtained for evaluation of phytotoxicity.

4. Report items

(1) Whether or not there is phytotoxicity; if so, their nature, degree (measurements of plant height, etc.), extent of recovery, etc.

(2) Other items

Information regarding crops (growth details, growth stage at the time of treatment, etc.)

Studies of residual odor in tea (1-1-3)

1. Objective

The objective of these studies is to obtain scientific information as to whether or not there is an odor brought about by the agricultural chemical remains as a side effect of using it on tea plants.

2. Test crops

Use healthy tea plants cultivated according to the usual methods. Use the *Yabukita* variety of tea.

3. Study methods

(1) Conduct the study with the methods and dosage (or concentration) relevant to the application for registration, and establish an untreated section.

(2) Conduct the study according to methods by which sufficient information will be obtained for evaluation of phytotoxicity.

4. Report items

(1) Whether or not there is a residual odor

(2) Other items

(i) Information regarding crops (growth details, growth stage at the time of treatment, etc.)

(ii) Information regarding tea processing, storage, etc.

Tobacco taste studies (1-1-4)

1. Objective

The objective of these studies is to obtain scientific information as to whether or not there is an effect on flavor brought about by the agricultural chemical remains as a side effect of using it on tobacco.

2. Test crops

Use healthy tobacco plants cultivated according to the usual methods. Select a representative variety.

3. Study methods

(1) Conduct the study with the methods and dosage (or concentration) relevant to the application for registration, and establish an untreated section.

(2) Conduct the study according to methods by which sufficient information will be obtained for evaluation of tobacco flavor.

4. Report items

(1) Whether or not there is an effect on flavor

(2) Other items

(i) Information regarding crops (growth details, growth stage at the time of treatment, etc.)

(ii) Information regarding tobacco processing, storage, etc.

Studies of surrounding crop phytotoxicity (1-2-1~3)

Studies of phytotoxicity due to drift and scattering (1-2-1)

1. Objective

The objective of these studies is to obtain scientific information regarding surrounding crop phytotoxicity due to scattering of the agricultural chemical.

2. Test crops

(1) As regards varieties of test crops, care should be taken to select at least 1 representative variety each from among such crops as Solanaceae, Cucurbit, Brassicae, Legume, and Gramine according to the type of crop and area to which the chemical is being applied.

(2) Use representative crop varieties, at the stage of growth of maximum susceptibility.

3. Study methods

(1) Conduct the study with the methods and dosage (or concentration) relevant to the application for registration, and establish an untreated section.

(2) Conduct the study according to methods by which sufficient information will be obtained for evaluation of phytotoxicity.

4. Report items

(1) Whether or not there is phytotoxicity; if so, their nature, degree (measurements of plant height, etc.), extent of recovery, etc.

(2) Other items

Information regarding crops (growth details, growth stage at the time of treatment, etc.)

Studies of phytotoxicity due to runoff from paddy water (1-2-2)

1. Objective

The objective of these studies is to obtain scientific information regarding side crop phytotoxicity, etc. that grow in water systems, with reference to agricultural chemicals, among those that are applied to rice paddies, that run off into water systems via paddy water.

2. Test crops

Use representative varieties of such crops as rush, lotus root, and arrow head bulb. Use representative crop varieties, at

the stage of growth of maximum susceptibility.

3. Study methods
(1) Conduct the study with the methods and dosage (or concentration) relevant to the application for registration, and establish an untreated section.
(2) Conduct the study according to methods by which sufficient information will be obtained for evaluation of phytotoxicity.

4. Report items
(1) Whether or not there are phytotoxicity; if so, their nature, degree (measurements of plant height, etc.), extent of recovery, etc.
(2) Other items
Information regarding crops (growth details, growth stage at the time of treatment, etc.)

Studies of phytotoxicity due to volatilization (1-2-3)

1. Objective
The objective of these studies is to obtain scientific information regarding surrounding crop phytotoxicity due to volatilization from water or soil of agricultural chemicals (herbicides only) especially with highly active in trace amount among chemicals which the active ingredient is a substance that has a high vapor pressure and low aqueous solubility.

2. Test crops
Use representative crops that are likely to be highly susceptible. Use representative crop varieties, at the stage of growth of maximum susceptibility.

3. Study methods
(1) Conduct the study with the methods and dosage (or concentration) relevant to the application for registration, and establish an untreated section.
(2) Conduct the study according to methods by which sufficient information will be obtained for evaluation of phytotoxicity.

4. Report items
(1) Whether or not there is phytotoxicity; if so, their nature, degree (measurements of plant height, etc.), extent of recovery, etc.
(2) Other items
Information regarding crops (growth details, growth stage at the time of treatment, etc.)

Studies of succeeding crop phytotoxicity

Succeeding crop phytotoxicity studies (1-3)

1. Objective
The objective of these studies is to obtain scientific information regarding phytotoxicity on succeeding crops of agricultural chemicals that are persist in soil for a long period and are deemed necessary due to the cultivation period of the target crop, etc., among soil treatment formulation or agricultural chemicals which have concern to be mixed with soil.

2. Test crops
Select crops that are considered to be highly susceptible from crops which have a possibility to be cultivated as

succeeding crops of the target crop. Use representative crop varieties at the stage of growth of maximum susceptibility.

3. Study methods
(1) Conduct the study with the methods and dosage (or concentration) relevant to the application for registration, and establish an untreated section.
(2) Conduct the study according to methods by which sufficient information will be obtained for evaluation of phytotoxicity.

4. Report items
(1) Whether or not there is phytotoxicity; if so, their nature, degree (measurements of plant height, etc.), extent of recovery, etc.
(2) Other items
Information regarding crops (growth details, growth stage at the time of treatment, etc.)

< Toxicity Studies >

Acute oral toxicity studies (2-1-1)

1. Objectives

These studies are the first step in evaluating the toxicity of agricultural chemicals. Their objective is to establish safe methods of handling agricultural chemicals when they are used, by obtaining scientific knowledge as to injuries to health that may result from a single oral exposure. The studies are also useful for obtaining initial scientific knowledge of the test substance's properties as regards toxic effects that will be of use in setting dosages for repeated dose toxicity studies and other studies.

2. Test method

Acute oral toxicity test methods include the fixed dose method and the toxic class method, among others.

I. Fixed dose method

1. Test animals
(1) Use young adult rodents (usually rats).
(2) In principle, use females. However, males should be used when there is information indicating that males are more sensitive to the test substance.
(3) Use females that are nulliparous and non-pregnant.

2. Administration method

Administer the test substance in a single dose by gavage, and when necessary, dissolve or suspend it in water or a suitable vehicle. However, the relevant vehicle should be of known toxicity and such as will not seriously affect the test results.

The species of animal used should be considered as regards the degree of fasting prior to test substance administration.

3. Observation period

Observations should be conducted for at least 14 days.

4. Setting the number of animals
(1) Sighting study
Use 1 animal for each dose level.
(2) Main study

Use 5 animals for each dose level. However, for dose levels that have been implemented in the sighting study, 4 animals are added to the 1 animal used in the sighting study, for a total of 5 animals.

5. Test procedure

(1) Sighting study

To select the starting dose levels for the main study, conduct the study in accordance with Annex 2-1-1-① using the doses of 5, 50, 300, and 2,000 mg/kg of body weight. As the initial dose level, select a dose at which evident toxicity is expected to be manifested. When no information is available regarding the acute toxicity of the test substance, it is desirable to start with a dose of 300 mg/kg. A period of at least 24 hours should be allowed between the dosing of each level.

When death occurs at a dose level of 5 mg/kg, regard LD_{50} as $\leqq$ 5 mg/kg of body weight, and terminate the study without conducting the main test. However, if further confirmation of LD_{50} is needed, additional procedures may be conducted.

(2) Main study

Conduct the study in accordance with Annex 2-1-1-②. However, regard the dose level at which a death is observed in the sighting study as one in which 2 deaths occur in the main study, without actually conducting the main study. Determine the interval between dosing at each level according to the duration and severity of toxic symptoms. Do not proceed to the next dose until survival or death can be confirmed for the previous dosed animals.

(3) Limit test

If there are no deaths in the sighting study at a dose level of 2,000 mg/kg, and 1 death or none in the main test at a dose level of 2,000 mg/kg, there is no need to administer doses exceeding 2,000 mg/kg.

6. Observation and examination

Conduct the items in (1) and (2) below.

(1) Observation as to general condition

(i) Carefully observe the general condition of animals at least once within 30 minutes after dosing, frequently during the following day, and thereafter at least once daily.

(ii) Keep a record of all types of symptoms of poisoning noted by gross observation in each animal, as well the time of occurrence, and the time of recovery or death.

(iii) Weigh the test animals immediately prior to and 1 week after test substance administration. If a test animal dies, weigh it at the time of death.

(iv) In order to minimize the loss of test animals that are useful for evaluating toxicity, institute appropriate measures (necropsy with gross observation, quarantining, etc.) promptly upon discovering dead, weakened, or moribund animals.

(2) Pathological examination

Conduct necropsies of all test animals, and record gross pathological findings. It is desirable to conduct histopathological examinations with reference to macroscopic gross observations of the organs of test animals that survived 24 hours or more after test substance administration.

II. Toxic class method

1. Test animals

As per fixed dose method.

2. Administration method

As per fixed dose method.

3. Observation period

As per fixed dose method.

4. Setting the number of animals

Use 3 animals for each dose step.

5. Test procedure

(1) Select the starting test dose levels from among dosages of 5, 50, 300, and 2,000 mg/kg of body weight, and conduct tests in accordance with Annex 2-1-1-③. As the initial dose level, select a dose which is most likely to produce mortality in some of the dosed animals. When no information is available regarding the acute toxicity of the test substance, it is desirable to start with a dose of 300 mg/kg.

(2) Determine the interval between dosing at each step according to the duration and severity of toxic symptoms. Do not proceed to the next dosing until survival or death can be confirmed for the previous dosed animals.

(3) If there is 1 death or none at a dose level of 2,000 mg/kg administered to 3 animals, 2,000 mg/kg should be administered to an additional 3 animals. If the test substance causes 1 or no deaths after the second administration as well, there is no need to administer doses exceeding 2,000 mg/kg.

6. Observation and examination

Conduct the items in (1) and (2) below.

(1) Observation as to general condition

As per fixed dose method.

(2) Histological studies

As per fixed dose method.

Acute Dermal Toxicity Studies (2-1-2)

1. Objective

The objective of these studies is to establish safe methods of handling agricultural chemicals when they are used, by obtaining scientific information regarding health hazards that may result from a single dermal exposure to the agricultural chemical.

2. Test animals

(1) Use 1 or more species of mammals such as rats, rabbits, or guinea pigs. (Generally speaking, the weight ranges of test animals should be as follows: Rats, 200–300 g; rabbits, 2.0–3.0 kg; guinea pigs 350–450 g.)

(2) Use young adults.

(3) Nulliparous, non-pregnant females should be used.

3. Administration method

(1) Remove the hair from the back trunks of the test animals by clipping or shaving 24 hours prior to test substance administration. At this time, be careful to avoid injuring the skin, since this will affect its permeability to the test substance.

(2) Shave completely at least 10% of the body surface area (rats, 4 cm x 5 cm; rabbits, 12 cm x 14 cm; guinea pigs, 7 cm x 10 cm) for application of the test substance. Take the animals' body weights into account when determining the area to be shaved.

(3) Apply the test substance within a range that is approximately 10% of the body surface area. There are cases in which test substances of high toxicity may be applied to a smaller area, but insofar as possible, apply the substance thinly and uniformly to the entire application site.

(4) When the test substance is a solid, grind it as appropriate, and moisten it with water or other vehicle so that it makes good contact with the skin. If using a vehicle, use one that will not irritate the skin, and be careful that the vehicle does

not affect the skin's permeability to the test substance.

(5) The test substance is to be applied to the skin for a period of 24 hours. During that time, cover the application site with porous gauze, and secure it with non-irritating tape so as to preserve contact with the skin. An additional appropriate method of covering must be used to preserve the test substance and gauze, so that the test animals are not able to ingest the test substance.

(6) Use water or an appropriate vehicle to remove test substance that is adhering to the skin at the end of the application period.

4. Observation period

Conduct observations for at least 14 days.

5. Determining the number of animals, and establishing test groups

(1) Determining the number of animals

Assign 5 animals, all of the same sex, to each group.

(2) Establishing test groups

(i) Establish test substance dosage groups according to at least 3 dosage levels.

(ii) In addition to studies on one sex, administer the substance to at least 1 group of the other sex, to confirm that the other sex of the animal does not have a notably high susceptibility to the test substance. If sufficient information has been obtained to indicate that one sex is more susceptible to the test substance than the other, studies with the other sex may be omitted.

(iii) Establish groups with dosage levels at appropriate intervals for symptoms of poisoning and death among test animals. The groups must be established so as to be sufficient for determination of the dose-response curve and LD_{50}.

(3) Limit tests

If death is not confirmed as a result of a single administration of 2,000 mg/kg of body weight or more of the test substance, it is not necessary to conduct studies with groups that receive a higher dosage than that. However, 1 group of the opposite sex should also be administered 2,000 mg/kg of body weight, in order to check susceptibility.

6. Observation and examination

Conduct the items in (1) and (2) below.

(1) Observation as to general condition

(i) Carefully observe the general condition of animals, frequently during the day on which the test substance is administered, and thereafter at least once daily.

(ii) Keep a record of all types of symptoms of poisoning noted by gross observation in each animal, as well the time of occurrence, and the time of recovery or death.

(iii) Weigh the test animals immediately prior to and 1 week after test substance administration. If a test animal dies, weigh it at the time of death.

(iv) In order to minimize the loss of test animals that are useful for evaluating toxicity, institute appropriate measures (necropsy with gross observation, quarantining, etc.) promptly upon discovering dead, weakened, or moribund animals.

(2) Pathological examination

As per acute oral toxicity studies.

Acute Inhalation Toxicity Studies (2-1-3)

1. Objective

The objective of these studies is to establish safe methods of handling agricultural chemicals when they are used, by obtaining scientific information regarding health hazards that may result from a single exposure to the agricultural

chemical via inhalation.

2. Test animals
   (1) Use young adult mammals (usually rats) of 1 or more species.
   (2) Nulliparous, non-pregnant females should be used.

3. Exposure method
   (1) Expose animals to the set concentration of the substance for at least 4 hours, using inhalation equipment. The exposure method may be whole body or pernasal exposure. Do not provide food or water during exposure.
   (2) Monitor flow rate, actual concentration of the test substance, particle size distribution, temperature, and humidity during exposure. Maintain the uniformity of these conditions.
   (3) Particle size (aerodynamic mass median size) of 1-4 μm is preferable. Or, use the minimum particle size with which it is possible to conduct the test.
   (4) If the test substance is volatile, ensure that it is not of a concentration that would cause an explosion.

4. Observation period
   Conduct observations for at least 14 days.

5. Determining the number of animals, and establishing test groups
   (1) Determining the number of animals
   Assign 5 animals, all of the same sex, to each group.
   (2) Establishing test groups
   (i) Establish test substance dosage groups according to at least 3 dosage levels.
   (ii) In addition to studies on one sex, administer the substance to at least 1 group of the other sex, to confirm that the other sex of the animal does not have a notably high susceptibility to the test substance. If sufficient information has been obtained to indicate that one sex is more susceptible to the test substance than the other, studies with the other sex may be omitted.
   (iii) Establish groups with exposure levels at appropriate intervals for symptoms of poisoning and death among test animals.
   (iv) The groups must be established so as to be sufficient for determination of the concentration/response relationship, and the approximate median lethal dose (LC_{50}).
   (v) When a vehicle is used for maintaining the proper concentration of test substance in the exposure environment, it is desirable not to use a vehicle that is known to be toxic, or that will greatly affect the study results.
   (vi) When necessary, conduct studies with a vehicle control group.
   (3) Limit test
   (i) If death ascribable to the test substance does not result in studies with an exposure concentration of 5 mg/L for 4 hours, it is not necessary to conduct studies with higher concentrations than that. However, 1 group of the opposite sex should also be exposed to a concentration of 5 mg/l, in order to check susceptibility.
   (ii) When an exposure concentration of 5 mg/L is impossible due to the physico-chemical properties of the test substance, and no death that is ascribable to the test substance occurs at the maximum concentration that can be obtained by means of the operational procedures in this study method, it is not necessary to conduct studies with higher concentrations than that. However, 1 group of the opposite sex should also be exposed to a high concentration, in order to check susceptibility.

6. Observation and examination
   Conduct the items in (1) and (2) below.
   (1) Observation as to general condition
   As per dermal toxicity tests.
   (2) Pathological examination

(i) Pay attention to changes in the respiratory system, and take observed instances of poisoning into account. Conduct necropsies of all test animals, and record macroscopically pathological findings.

(ii) It is desirable to conduct histopathological examinations with reference to macroscopical gross observations of the organs of test animals that survived 24 hours or more.

Skin irritation studies (2-1-4)

1. Objective

The objective of these studies is to establish safe methods of handling agricultural chemicals when they are used, by obtaining scientific information regarding skin irritant properties and corrosiveness of the test substance.

2. Test animals

Use 3 or more young adult albino rabbits.

3. Administration method

(1) Crip the hair on the back trunks of the animals 24 hours prior to the study. Be careful not to injure the skin, and only use healthy animals with undamaged skin.

(2) When the test substance is a solid, grind it, as appropriate, and moisten it with water or other vehicle, so that it makes good contact with the skin. If using a vehicle, use one that will not irritate the skin, and be careful that the vehicle does not affect its permeability by the test substance. In general use liquid test substance undiluted.

(3) Apply locally 0.5 ml of liquid substance, or 0.5 g of solid test substance in the form of a paste.

(4) Apply the test substance to a small area of the skin (approximately 6 cm^3). During the administration (application) period, cover the site with a gauze patch, secured with non-irritating tape. Procedures whereby the test substance, in the form of a liquid or paste, is applied to the gauze patch, which is then applied to the skin, are also acceptable. Using a semi-occlusive dressing, situate the patch in such a way that it will maintain contact with the skin throughout the exposure period (in some cases, an occlusive dressing may be used instead). Compare to the untreated areas of the animal's body.

(5) Normally, the exposure period should be 4 hours in duration. Use water or an appropriate vehicle to remove test substance that is adhering to the skin at the end of the exposure period.

4. Points to keep in mind regarding application

(1) When severe skin irritant properties or corrosiveness are suspected:

(i) Conduct the test with only 1 animal when it is suspected that the test substance has severe skin irritant properties or corrosiveness.

(ii) When corrosiveness is suspected, apply 3 test patches simultaneously to 1 animal. Remove the first patch after an exposure of 3 minutes. If no severe skin reaction is observed, remove the second patch after an exposure of 1 hour. If it is found at this stage, from the standpoint of prevention of cruelty to animals, that the study period can be extended to 4 hours, remove the third patch after 4 hours of exposure. If severe skin irritation is observed after 3 minutes or 1 hour of exposure, remove the remaining patch(es) immediately.

Instead of the above, the 3 patches may be applied successively, and observations made accordingly.

(iii) When it is suspected that the test substance is a severe skin irritant, apply 1 patch to 1 animal for 4 hours.

(iv) If no severe skin irritation is observed after a 4-hour exposure, 2 other animals may be tested with the patch for 4 hours each.

(2) When the test substance is not expected to cause severe skin irritation or corrosion:

Commence studies using 3 animals, applying to each 1 patch for 4 hours of exposure.

5. Observation and rating of general condition

(1) Examine symptoms of erythema and edema when patches have been removed from the animals after 30 (or 60)

minutes, 24 hours, 48 hours, and 72 hours. Rate the skin reactions.

(2) Rate and record skin irritation properties and corrosiveness according to the criteria in the table below. Continue observations thereafter when it is necessary to clarify whether the symptoms are reversible. In general, it is not necessary for observations to extend beyond 14 days.

(3) Keep proper records of severe injuries and other signs of poisoning, in addition to skin irritation and corrosion.

(Table) Skin irritation and corrosion criteria

1. Erythema and eschar formation

(1) No erythema 0
(2) Very slight erythema (barely perceptible) 1
(3) well-defined clear erythema 2
(4) Moderate to severe erythema 3
(5) Severe erythema (deep redness) or eschar formation (erythema is not scorable) 4

Maximum score: 4

2. Edema formation

(1) No edema 0
(2) Very slight edema (barely perceptible) 1
(3) Slight edema (edges ofarea well definded be definite raising) 2
(4) Moderate edema (swelling of approximately 1 mm) 3
(5) Severe edema (swelling $\geq$ 1 mm, and extending beyond the exposed area) 4

Maximum score: 4

Eye irritation studies (2-1-5)

1. Objective

The objective of these studies is to establish safe methods of handling agricultural chemicals when they are used, by obtaining scientific information regarding irritation and corrosion of eyes and ocular mucous membranes by the test substance.

2. Test animals

Use 3 or more young adult albino rabbits.

3. Administration method

(1) Examine both eyes of test animals within 24 hours prior to study commencement. Do not use animals with ocular abnormalities.

(2) Apply no more than 0.1 ml of undiluted liquid test substance, or 0.1 ml by volume or 0.1 g by weight of paste. (Always record volume or weight.) If the test substance is solid or granular, grind it to a fine powder.

(3) Gently separate the lower eyelid of one eye from the eyeball, and apply the test substance to the conjunctival sac. Gently bring upper and lower eyelids together for approximately 1 second, in order to prevent loss of test substance. Compare with the other, untreated eye.

Apply test substance that comes in pressurized aerosol containers by spraying a single jet for 1 second from 10 cm in front of the open eye.

(4) Local anesthetic may be used if it is thought that the test substance will cause severe pain. However, sufficient care should be taken that use of the local anesthetic does not lead to a significant difference in the organism's reactivity to the test substance.

(5) Do not wash the eye to which the test substance was applied for 24 hours following administration of test substance eye drops. The eye may be washed 24 hours after administration if this is deemed appropriate.

(6) If severe ocular irritation occurs as a result of the test substance, conduct studies on at least 3 animals as to the effectiveness of washing their eyes. In such cases, wash the treated eye 30 seconds after administration for 30 seconds in a volume and at a flow velocity that will not injure the eye.

4. Points to keep in mind regarding application

Conduct the study with only 1 animal when it is suspected that the test substance is a severe eye irritant. If severe eye irritation or corrosion are observed as a result, it is not necessary to conduct studies with additional test animals.

5. Observation of general condition and scoring

(1) Observe the general condition of the eye 1 hour, 24 hours, 48 hours, and 72 hours after administration. Record observations, and also record reactivity (irritation or corrosion) of the eye to the test substance, based on the criteria in the table below. Fluorescein may be used in some or all of the test animals' eyes following examination 24 hours after administration, and examinations repeated.

(2) If no eye irritation is observed by 72 hours after administration, the studies can be regarded as finished.

(3) If persistent corneal injury or other ocular irritation is noted, continue to observe its progress (as to reversibility, irreversibility, etc.) for no more than 21 days following administration of the test substance.

(4) In addition to observation of the cornea, iris, and conjunctiva, keep records of all injuries, etc. that are observed.

(Table) Eye irritation and corrosion criteria

Cornea *

Opacity: Degree of opacity (determined according to the most opaque area)

(1) No ulcers or opacity observed 0

(2) Sporadic or diffused opacity (different from the degree of cloudiness having ordinary luster); the details of the iris are clearly translucent .....

(3) There are some clear areas left, but nearly all of the iris is obscured 2

(4) Nacreous areas, no details of iris visible, size of pupil barely discernible 3

(5) Corneal opacity; iris not discernible through opaque areas 4

Maximum score: 4

* Record the area of corneal opacity

Iris

(1) Normal 0

(2) Clear and deep frugae, congestion, swelling, moderate hyperemia in the corneal periphery; any of these singly or in combination; the iris still reacts to light (reaction is slow and dull) 1

(3) No reaction to light; hemorrhage; gross destruction (any or all of these) 2

Maximum score: 2

Conjunctiva

Redness (eyelid, bulbar conjunctiva, cornea, and/or iris)

(1) Blood vessels normal 0

(2) Clear hyperemia in some blood vessels 1

(3) Diffuse crimson; individual blood vessels cannot be readily discerned 2

(4) Diffuse beefy red 3

Maximum score: 3

Conjunctival edema (palpebral conjunctiva and/or nictitating membrane)

(1) No swelling 0

(2) Greater than normal swelling(including nctating membranes) 1

(3) Obvious swelling accompanying ectropion of the eyelid 2

(4) Swelling such that the eyelid is less than half 3
(5) Swelling such that the eyelid is half or more 4
Maximum score: 4

Skin sensitization studies 2-1-6

1. Objective

The objective of these studies is to establish safe methods of handling agricultural chemicals when they are used, by obtaining scientific information regarding skin sensitization by the test substance.

2. Test animal species, age, and sex

(1) Use young adult guinea pigs.
(2) Nulliparous, non-pregnant females should be used.

3. Study methods

The Guinea Pig Maximization Test (hereinafter referred to as the "GPM method") and the Buehler Test (hereinafter referred to as the "Buehler method") are study methods that are conducted relatively frequently. However, other study methods may be substituted if information regarding sensitization can be obtained thereby.

4. Study Procedures

(1) GPM method

(i) Establishing test groups

Establish a test substance treatment group, a negative control group, and a positive control group. Conduct studies with the positive control group, using substances known to sensitize. Recent background data may be used if available.

(ii) Establishing the number of animals

a. There should be at least 10 animals in each test substance treatment group, and at least 5 in each control group.
b. If there are less than 20 animals in each test substance treatment group, and less than 10 animals in each control group, and it cannot be concluded whether the test substance causes sensitization, it would be desirable to conduct additional studies in which there are at least 20 animals in each test substance treatment group, and at least 10 animals in each control group.

(iii) Establishing dosages

a. The concentration of test substance used for induction exposure should be such as the test animal is, in general, sufficiently able to tolerate, and should be the maximum concentration at which slight to moderate skin irritation occurs.
b. The concentration of test substance used for challenge exposure should be the maximum at which irritation does not occur.
c. Use 2 or 3 animals, and determine the appropriate concentration of test substance.

(iv) Initial induction (intracutaneous injection)

Use the following method.

a. Test substance treatment group

Administer the following set of 3 injections (0.1 ml) on both sides of the median line of the area of the back from which the hair has been removed.

Injection 1: Freund's complete adjuvant (hereinafter referred to as "FCA") and water (or saline) in 1 : 1 (v/v) mixture
Injection 2: The designated concentration of test substance in an appropriate vehicle
Injection 3: The designated concentration of test substance in a 1 : 1 (v/v) mixture of FCA and water(or saline)

b.Negative control group

Administer the following set of 3 injections (0.1 ml) to the same site as in the test substance treatment group.

Injection 1: FCA and water (or saline) in a 1 : 1 (v/v) mixture

Injection 2: Only the vehicle used in the treatment group

Injection 3: FCA and water (or saline) in a 1 : 1 (v/v) mixture

(v) Re-induction (application by affixing to skin)

a.Re-induction 5-7 days after initial induction

If the test substance is not a skin irritant, apply 0.5 ml of petrolatum containing 10% sodium lauryl sulfate to the test site, on which the hair has been trimmed short or shaved approximately 24 hours prior to re-induction, in order to promote re-induction.

b.Re-induction 6-8 hours after initial induction.

Use the following method.

(a) Test substance treatment group

Remove the hair once again from the test site. Apply the test substance, which has been prepared with an appropriate vehicle, to the test site with filter paper or gauze sufficiently soaked in the preparation, and affix an occlusive dressing for 48 hours. It is necessary to have a reason for the vehicle selected. Grind solid test substance and mix it with an appropriate vehicle. Apply liquid test substance undiluted, when appropriate.

(b) Negative control group

Apply vehicle only, but in the same manner as with the treatment group, and affix an occlusive dressing for 48 hours.

(vi) Initial challenge (application by affixing to skin)

a. Conduct 14 days after re-induction (apply by affixing to skin).

b. Remove the hair from the ventral areas of animals in the teat substance administration and control groups. Affix a patch or chamber, to which the test substance has been applied, to one side of the animals' ventral areas, and, if necessary, affix another patch or chamber, to which only vehicle has been applied, to the other side in the same way.

c.Apply an occlusive dressing to the patch for 24 hours.

(vii) Observations

a.If necessary, remove the hair from the challenge area 21 hours after removing the pouch.

b.Note skin reactions 3 hours later (approximately 48 hours from the commencement of induction patch application), and record observations according to the rating standard shown below.

c. Observe skin reaction again 24 hours after the first observation, and record findings.

< Induction patch test reaction evaluation criteria >

No visible change 0

Diffuse or patchy erythema 1

Moderate dispersed erythema 2

Severe erythema and edema 3

(viii) Re-challenge

If it is necessary to further confirm results obtained from the initial challenge, re-challenge may be conducted 1 week after the initial challenge, after establishing a new control group, as appropriate. The control group used for the initial challenge may be used again.

(ix) Observations of general condition

Record all skin reactions and abnormal findings resulting from induction and challenge.

(2) Buehler method

(i) Establishing test groups

Establish a test substance treatment group, a negative control group, and a positive control group. Conduct studies with the positive control group, using substances known to sensitize. Recent background data may be used if available.

(ii) Establishing the number of animals

Use 20 animals in the test substance treatment group, and 10 in the control group.

(iii)Establishing dosages

Same as with the GPM method.

(iv) Initial induction (application by affixing to skin)

Use the following method.

a. Test substance treatment group

Remove the hair from one shoulder or side. Apply a test patch containing the test substance, which has been prepared with an appropriate vehicle, to the site, and secure it with an occlusive dressing for 6 hours.

b. Negative control group

Apply vehicle only, but otherwise use the same method as with the test substance treatment group.

(v) Re-induction (application by affixing to skin)

a. Conduct 6-8 days and 13-15 days after the initial induction

b.Treat in the same way the same shoulder or side used in the initial induction.

(vi)Challenge

a.Conduct 14 days after re-induction.

b.Remove the hair from the ventral areas of animals in the teat substance administration and control groups. Affix a patch or chamber, to which the test substance has been applied, to one side of the animals' ventral areas, and, if necessary, affix another patch or chamber, to which only vehicle has been applied, to the other side in the same way. Affix the patch with an occlusive dressing for 6 hours.

(vii) Observations

a.If necessary, remove the hair from the challenge area 21 hours after removing the patch.

b.Note skin reactions 3 hours later (approximately 30 hours from the commencement of induction patch application), and record observations according to the scoring system shown in the GPM method.

c. Observe skin reaction again 24 hours after the first observation, and record findings.

(viii) Re-challenge

Same as in the GPM method.

(ix)Observation of general condition.

Same as in the GPM method.

Acute neurotoxicity studies (2-1-7)

1. Objective

The objective of these studies is to establish safe methods of handling agricultural chemicals when they are used, by clarifying the neurotoxic properties of the relevant agricultural chemical following a single exposure, and by obtaining scientific information regarding the maximum dosage at which no toxic changes are observed (no observed adverse effect level: NOAEL).

2. Test animals

(1) Use rodents (rats, usually).

(2) Normally, use, as soon as possible after weaning and following acclimatization period, animals that are the same age, 5-6 weeks old.

(3) In general, use the same number of male and female animals. Nulliparous and non -pregnant females should be used.

3. Administration method

(1) Select the method of administration (oral, dermal, or inhalation) as necessary.

(2) Use the same methods as in the acute oral toxicity studies, the acute dermal toxicity studies, or the acute inhalation toxicity studies.

4. Observation period

Observe animals for 14 days following single exposure to the test substance.

5. Determining the number of animals, and establishing test groups

(1) Determining the number of animals

(i) Use 10 or more each of male and female animals in each group for the studies, as necessary for detailed observations of symptoms and examination of functions. From among these, select 5 or more each of male and female animals in each group as necessary for neurohistopathological examination.

(ii) Use an appropriate random sampling method, by body weight differential, etc., for allocating animals to groups.

(iii) It is essential, ultimately, to ensure that there is a sufficient number of animals for evaluation of test results.

(2) Establishing test groups

(i) Establishing test substance administration groups

a. Establish test substance dosage groups according to at least 3 dosage levels.

b. Establish dosage levels such that symptoms of test substance toxicity will be clear, and so that the NOAEL can be estimated. As regards maximum dosages, set each dose level such that the dosage at which toxic effects not resulting in death and the minimum dose at which no toxic effects can be determined, and so that the relationship between dosages and reactions can be discerned.

c. Refer to the results of previously conducted toxicity studies when establishing dosages. In addition, indicate the grounds for the dosages established.

d. If death is not confirmed as a result of a single administration of the maximum dose that is technically possible, or 2,000 mg/kg of body weight of the test substance, it is not necessary to conduct studies with higher dosages than that.

(ii) Control group

a. Establish the same conditions for the control group as for the test substance administration groups, except that the test substance is not administered.

b. When a vehicle, etc. is being used for administration of the test substance, administer to the control group the same amount of vehicle as to the dosage group that is being administered the largest amount of vehicle. Establish an additional untreated control group when using a vehicle, etc. regarding which sufficient information on toxicity cannot be obtained.

6. Observation and examination

Conduct items (1)-(4) below.

(1) Observations as to general condition

(i) Carefully observe the general condition of the animals every day.

(ii) Observe overall condition, and whether or not any abnormal behavior or deaths occur.

(iii) Weigh all animals prior to the commencement of administration, and at least 1 week after the commencement of administration.

(2) Detailed observation of condition

(i) Conduct these observations in regard to 10 or more each of male and female animals in each relevant group prior to the commencement of administration, and 8 hours after administration, when the effect is expected to be most pronounced, as well as 7 and 14 days after administration. Observe the animals both in their cages and on the observation stand.

(ii) Use rating methods for which the criteria for judgement and measurement have been clearly stipulated, and follow standardized procedures.

(iii) In general, conduct examinations in regard to the following items:

Appearance (skin; fur; changes in eyes, eyeballs, and mucous membranes), body position and posture (hunchback posture, etc.), autonomic nervous system function (lacrimation, piloerection, pupil diameter, respiration, excretion, etc.), motor coordination, ambulatory abnormalities, animal's reactions to being handled and

to environmental stimulation, nervous system (tremor, convulsion, muscular contractions, etc.), changes in exploratory behavior, ordinary behavior (changes in grooming, headshaking, gyration, etc.), abnormal behavior (autophagia, backward motion, abnormal vocalization, etc.), aggression, etc.

(3) Functional examination

(i) Conduct these observations in regard to 10 or more each of male and female animals in each relevant group prior to the commencement of administration, and 8 hours after administration, when the effect is expected to be most pronounced, as well as 7 and 14 days after administration.

(ii) In general, conduct examinations in regard to the following items:

Sensorimotor reactions to various stimuli (including auditory, visual, and proprioceptor stimuli), grip strength, and amount of spontaneous motor activity (using an automatic recording apparetus).

(iii) If it is suspected, on the basis of other toxicity studies, that the test substance is a neurotoxin, conduct studies and careful examinations regarding the appropriate sensory mechanisms, motor function, and learning/memory relating to the suspected neurotoxicity.

(4) Physiological examinations

(i) Conduct histopathological examinations in regard to 5 or more each of male and female animals in each relevant group.

(ii) If abnormalities are noted in specific animals during symptomatic observations and functional examinations, examine these animals.

(iii) Fix tissues by means of perfusion fixation or other appropriate method. Record all gross observations of physiological changes.

(iv) In general, embed tissue sample in paraffin, and use a staining agent, such as hematoxylin and eosin stain. However, if nerve damage in the peripheral nervous system is observed or suspected, adjust and examine peripheral nerve tissues embedded in resin.

(v) Conduct additional examinations and special staining, as appropriate, based on symptomatic observations.

(vi) Evaluate neurohistopathological findings by relating them to other toxicity test results and behavioral effects.

(vii) Conduct phased examinations of representative sections from the central and peripheral nervous systems.

(viii) First of all, compare sections from the maximum dosage group and the control group. If no neurohistopathological changes are observed in the maximum dosage group, no further examinations are necessary. If changes are observed in the maximum dosage group, examine sections from the median and minimum dosage groups, in that order.

(ix) In general, examine the following tissues:

The central cerebrum, including the prosencephalon and the hippocampus; the mesencephalon, cerebellum, pons, and medulla oblongata; the eyeball, including the optic nerve and retina; the cervical and lumbar enlargements of the spinal cord; the spinal ganglion, the ventral and dorsal roots of nerve fibers, the proximal sciatic nerve, the proximal tibial nerve (in the knee area), the gastrocnemius bifurcation of the tibial nerve, and the skeletal muscles (especially the gastrocnemius).

(x) The sections from the spinal cord and peripheral nerves should include both horizontal and vertical sections.

Acute delayed neurotoxicity studies (2-1-8)

1. Objective

The objective of these studies is to establish safe methods of handling agricultural chemicals when they are used, by obtaining scientific findings regarding agricultural chemicals that may be expected to have delayed neurotoxic properties based on test results in regard to acute toxicity and other toxicity, or on the correlation of their chemical structures with those of other substances known to exhibit delayed neurotoxicity.

2. Test animals

(1) Use female chickens of ordinary varieties and strains.

(2) It is desirable to use young adult chickens of standard size, 8-12 months old.
(3) Use healthy animals that have not had viral diseases or drug treatments that would affect actual test results, nor any ambulatory abnormalities.

3. Administration method
(1) Administer once, forcibly and orally.
(2) If the test substance is a liquid, administer it as liquid concentrate, or after dissolving in an appropriate vehicle.
(3) If the test substance is a solid, administer it in solution, insofar as possible.

4. Observation period
Conduct observations for 21 days following administration.

5. Determining the number of animals, and establishing test groups
(1) Determining the number of animals
(i) Use a number of animals in both the administration and control groups such that the 6 birds needed for biochemical studies, as well as 6 birds that can survive until the end of the studies and can be used in histopathological studies, will be available.
(ii) Use a number of animals the positive control group such that the 3 birds needed for biochemical studies, as well as 3 birds that can survive until the end of the studies and can be used in histopathological studies, will be available.
(2) Establishing test groups
(i) Test substance administration group
Set the highest dosage possible (maximum non-lethal dose), at which fatalities were not observed in preliminary studies.
Administer the maximum non-lethal dose of the test substance, with 2,000 mg/kg/day as the upper limit.
(ii) Control group
a. Establish the same conditions for the control group as for the test substance administration group, except that the test substance is not administered.
b. When a vehicle, etc. is being used for administration of the test substance, administer to the control group the same amount of vehicle as to the dosage group that is being administered the largest amount of vehicle. Establish an additional untreated control group when using a vehicle, etc. regarding which sufficient information on toxicity cannot be obtained.
(iii) Positive control group
Conduct studies with substances known to have delayed neurotoxic properties (for example, TOCP). Recent background data may be used if available.

6. Note regarding care
Keep the animals in a cage or pen that is large enough to allow the animals to walk around freely, and readily permit observation of their ambulatory status.

7. Observation and examination
Conduct the following items (1)-(3).
(1) Observation of general condition
(i) Commence observation of all animals immediately after administration. Carefully observe them several times per day for the next 2 days, and then at least once per day for the following 21 days, or until planned sacrifice.
(ii) Keep records of all symptoms of toxicity, including their type, time of occurrence, degree, and duration. Evaluate ataxia on the basis of criteria comprising at least 4 levels.
(iii) Animals used in pathological examinations should be brought out of their cages at least twice per week. Conduct forced movement at fixed times, in order to be able to observe even slight toxic effects.
(iv) Conduct gross pathological examinations of moribund animals after removing and sacrificing them.

(v) Weigh all animals once prior to and once 1 week after administration.

(2) Biochemical examinations

(i) Sacrifice 6 birds each chosen at random from the administration and control groups, and 3 birds from positive control group, 72 hours after administration of the test substance. Harvest their brains and lumbar spinal cords, and measure the neuropathy target esterase (NTE, also called neurotoxic esterase) activity.

Normally, sacrifice 3 birds each from the administration and control groups 24 hours and 48 hours after administration, and sacrifice 3 birds from the positive control group 24 hours after administration. Harvest the brains and lumbar spinal cords from each of these birds. If it is determined that excretion of the test substance is extremely slow, based on moderate symptoms of toxicity, it would be desirable to sacrifice 3 birds each two times between the 24th and, at the latest, the 72nd hour after administration, taking into account the optimal interval for detecting delayed neurotoxicity induction, and harvest their brains and lumbar spinal cords.

(ii) Measuring the acetylcholine esterase activity in the organs of the same animals in which NTE activity was measured would be helpful in evaluation.

(3) Pathological examinations

(i) Conduct gross pathological examinations of all animals sacrificed, either as planned or as circumstances required, including observation of the appearance of the brain and spinal cord.

(ii) Conduct studies of nerve tissue harvested from at least 6 surviving birds from each group when testing is complete.

(iii) Fix the tissue by means of perfusion fixation, or other appropriate method.

a. Include sections from the cerebellum (central longitudinal plane), medulla oblongata, spinal cord, and peripheral nerves.

b. Collect spinal cord sections from the upper cervical, mesothorax, and lumbo-sacral areas.

c. Collect proximal, distal, and bifurcating sections of the tibial nerve, and also harvest the sciatic nerve.

(iv) Stain sections of the myelin sheaths and the axons according to an appropriate procedure.

90-day repeated oral toxicity studies (2-1-9)

1. Objective

The objective of these studies is to obtain scientific information regarding toxic changes that occur following oral administration of the test substance, repeated for at least 90 days, as well as the maximum dosage at which toxic changes are not observed (NOAEL). These studies are also useful for obtaining information concerning establishment of dosages for carcinogenicity studies and 1-year repeated oral toxicity studies.

2. Test animals

(1) Conduct studies on 1 species of rodent (usually rats) and 1 species of non-rodent (dogs, usually).

(2) As regards rodents, use, as soon as possible after weaning and following acclimatization period (usually 5-6 weeks old), animals that are same age; as regards non-rodents (dogs), use animals that are 4-6 months old.

(3) In principle, use the same number of male and female animals. Use nulliparous, non-pregnant females.

3. Administration method

Carry out repeated oral administration, usually mixed with the animals' feed or water. However, gavage administration may be conducted if these methods of administration prove difficult.

4. Administration period

At least 90 consecutive days.

5. Determining the number of animals, and establishing test groups

(1) Determining the number of animals

(i) As regards rodents, use at least 10 males and 10 females per group; as regards non-rodents, use at least 4 males and 4 females per group.

(ii) Use an appropriate random sampling method, by body weight differential, etc., for allocating animals to groups. If interim sacrifice is anticipated, establish the additional number of animals required for this reason.

(iii)It is essential to ensure that there is a sufficient number of animals for evaluation of study results.

(2) Establishing test groups

(i) Establishing test substance administration groups

a. In addition to the control group, establish test substance dosage groups according to at least 3 dosage levels.

b. Establish dosage levels such that symptoms of test substance toxicity will be clear, and so that the NOAEL can be estimated. As regards maximum dosages, set each dose level such that the dosage at which toxic effects not resulting in death and the minimum dose at which no toxic effects can be determined, and so that the relationship between dosages and response can be discerned. (Indicate the grounds for establishment of dosages.)

c. If no toxic effects are confirmed as a result of administration of the maximum dose that is technically possible, or 1,000 mg/kg of body weight/day of the test substance, it is not necessary to conduct studies with higher dosages than that.

(ii) Control group

a. Establish the same conditions for the control group as for the test substance administration groups, except that the test substance is not administered.

b. When a vehicle, etc. is being used for administration of the test substance, administer to the control group the same amount of vehicle as to the dosage group that is being administered the largest amount of vehicle. Establish an additional untreated control group when using a vehicle, etc. regarding which sufficient information on toxicity cannot be obtained.

6. Observation and examination

Conduct the following items (1)-(5).

(1) Observation of general condition

(i) Observe all animals daily to determine their general condition (times at which symptoms of toxicity occur, and their degree), and mortality. In addition, weigh them regularly, and measure the food consumption. (When the test substance is administered in drinking water, also measure water consumption. Same below.)

(ii) In general, weigh the animals and measure the food consumption they ingest once prior to commencement of administration, and at least once per week following the commencement of administration. Also compute the amount of test substance ingested.

(2) Detailed observation of condition

(i) Conduct observations once prior to commencement of administration, and at least once per week following the commencement of administration.

(ii) Observe the animals both in their cages and on the observation stand. Observe the animals carefully, in accordance with an observation procedure in which observation items, as well as the definitions of these items, and the rating criteria and order of observation, have been established sufficiently for ascertaining changes in general condition. Be careful to ensure that the handling of animals at observation time does not affect test results.

(iii) In general, conduct examinations in regard to the following items:

External appearance (skin; fur; changes in eyes, eyeballs, and mucous membranes), body position and posture (hunchback posture, etc.), autonomic nervous system function (lacrimation, piloerection, pupil diameter, respiration, excretion, etc.), mortor coordination, ambulatory abnormalities, animal's reactions to being handled and to environmental stimulation, nervous system (tremor, convulsion, muscular contractions, etc.), changes in exploratory behavior, ordinary behavior (changes in grooming, headshaking, gyration, etc.), abnormal behavior (autophagia, backward motion, abnormal vocalization, etc.), aggression, etc.

(3) Functional examination

(i) Conduct examination of rodents near the end of administration.

(ii) In general, conduct examinations in regard to the following items:

Sensorimotor reactions to stimuli (including auditory, visual, and proprioceptor stimuli), grip strength, and amount of motor activity (measure using an automatic recording apparatus).

(4) Blood tests

(i) It would be desirable, in principle, to conduct blood tests of all animals, at least once at the end of the study, in the case of rodents, and both prior to and at the end of the study, in the case of non-rodents. However, in the case of rodents, the examinations may be limited a part of each group (at least 5 animals), for practical reasons.

(ii) Except in the case of mice, it would be desirable for animals to fast for 1 night prior to examination.

(iii) Usually, conduct examinations with regard to the following points. In addition to these, other items could be selected and added as appropriate. Make selections with regard to examination items and methods that are widely accepted internationally.

a. Hematological tests

Red blood cells, leukocyte cells, hemogram (percentage by leukocyte group), platelet count, hemoglobin, hematocrit; in addition to these, reticulocyte count, clotting capability, (prothrombin time, activated partial thromboplastin time) etc.

b. Blood biochemical tests

Serum (plasma) protein, albumin, A/G ratio, glucose, cholesterol, triglyceride, bilirubin, urinary nitrogen, creatinine, transaminase (AST (GOT), ALT (GPT)), γ-GTP, alkaline phosphatase, electrolytes (sodium, potassium, chlorine, calcium, inorganic phosphorus, etc.), etc.

(5) Urinalysis

(i) As regards rodents, conduct urinalyses of a fixed number (5 or more) of male and female animals in each group; as regards non-rodents, conduct urinalyses of all animals. Conduct urinalyses at the same time as blood tests.

(ii) Usually, conduct urinalyses with regard to the following points.

Urine volume, pH, protein, sugar, keton bodies, bilirubin, urobilinogen, occult blood, sediment, specific gravity, etc.

(6) Ophthalmological examination

Conduct ophthalmological examinations of all animals among non-rodents, and among at least the high dosage and control groups among rodents, prior to administration and at the end of the study. If abnormality is observed that are ascribable to the test substance, conduct examinations of all animals.

(7) Pathological examination, etc.

(i) Promptly conduct necropsies, as well as gross observations, of organs and tissues, and histopathological examinations, in cases of death during the administration period. Determine the cause of death, and the degree of toxic changes at the time of death.

(ii) Promptly sacrifice and dissect animals that become moribund during the test period; conduct observations and examinations as in (i) above. Determine the cause of moribundity, and the degree of toxic changes at the time the animal became moribund.

(iii) Sacrifice and dissect all surviving animals at the end of the administration period, after having collected blood and urine for the various tests. Conduct gross examinations of organs and tissues, and weigh each organ.

(iv) Usually, weigh the following organs. Except in the case of mice, it would be desirable for animals to fast for 1 night prior to examination.

Liver, kidneys, adrenal glands, testes, ovaries, thymus gland, spleen, heart, brain, prostate gland(NOTE), thyroid gland, parathyroid gland(NOTE), and pituitary gland(NOTE)

NOTE: Examine the prostate, parathyroid, and pituitary glands of non-rodents only.

(v) Conduct histopathological examinations of all animals among non-rodents, and at least of those in the high dosage and control groups among rodents.

(vi) Usually, conduct pathological examinations of the following organs and tissues, and add others as appropriate upon gross examination.

Skin, mammary glands, lymph nodes, (cervical and mesenteric lymph nodes, etc.), aorta, salivary glands, bones

and marrow (sternum and femur), thymus gland, trachea, lungs and bronchia, heart, thyroid and parathyroid glands, esophagus, stomach, small intestine (duodenum, jejunum, and ileum), large intestine (cecum, colon, and rectum), liver (and gallbladder), pancreas, spleen, kidneys, adrenal glands, bladder, seminal vesicle and coagulating gland, prostate, testes(NOTE), epididymis, ovaries, uterus, vagina, brain, pituitary gland, sciatic nerve, skeletal muscle, spinal cord (cervical, thoracic, and lumbar areas), eyeballs and appendages, and other organs and tissues in which changes can be confirmed with the gross.

NOTE: Examine the testes using a fixative, such as Bouin fixative, that is appropriate for retaining the structure of seminiferous tubules.

(vii) If, in dosage groups of animals other than rodents, there are organs in which changes due to administration of the test substance are observed with the gross examination, or if it is deemed necessary on the basis of findings in high dosage groups, conduct histopathological examinations of all animals in those groups. Among rodents, histopathological examination of all animals would be helpful in evaluations.

(viii) Even after the completion of studies, preserve organs and tissues so that histopathological examinations can be conducted if necessary.

7. Other

It would be desirable to conduct the following studies in cases in which effects on the nervous system, immune system, or endocrine system are confirmed on the basis of above-mentioned test results.

(1) Nervous system: repeated oral dose neurotoxicity studies

(2) Immune system: immunohistochemical staining of fresh frozen samples, measurement of splenic lymphocyte composition, measurement of natural killer (NK) cell activity, measurement of immunoglobulin (IgG, IgM, IgE, etc.) etc.

(3) Endocrine system: Measurement of steroid and thyroid hormones, etc. in the blood.

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

1. Objective

The objective of these studies is to establish safe methods of handling agricultural chemicals when they are used, by obtaining scientific information regarding toxic changes that occur following repeated dermal administration of the test substance, repeated for 21 days, as well as the maximum dosage at which toxic changes are not observed (NOAEL).

2. Test animals

(1) Use 1 or more species of mammals such as rats, rabbits, or guinea pigs.

(2) Use adult animals. In order to conduct the studies readily, it is desirable to stay within the following weight ranges: Rats, 200-300 g; rabbits, 2.0-3.0 kg; guinea pigs 350-450 g

(3) In general, use the same number of male and female animals. Use nulliparous and non-pregnant females.

3. Administration method

(1) Remove the hair of an appropriate site on the trunk of the animals immediately prior to the study. The hair may be shaved, but in this case do it 24 hours prior to the study.

In general the animals are shaved at 1-week intervals. Be careful in shaving not to injure the skin, since this may affect its permeability by the test substance.

(2) Remove the hair from approximately 10% of the total body surface area. Take body weight into account when determining the application area and area to be shaved.

(3) When the test substance is a solid, grind it to powder, as appropriate, and moisten it with water or other vehicle, so that it makes good contact with the skin. If using a vehicle, use one that will not irritate the skin, and be careful that the vehicle does not affect its permeability by the test substance. In general, use liquid test substance undiluted.

(4) Apply the test substance within a range that is approximately 10% of the body surface area (for example, with

animals within the above-mentioned weight ranges: rats, 4 cm x 5 cm; rabbits, 12 cm x 14 cm; guinea pigs, 7 cm x 10 cm). There are cases in which test substances of high toxicity may be applied to a smaller area, but insofar as possible, apply the substance thinly and uniformly to the entire application site.

(5) During the test substance application period, cover the application site with porous gauze, and secure it with non-irritating tape so as to preserve contact with the skin. An additional appropriate method of covering must be used to preserve the test substance and gauze, so that the test animals are not able to ingest the test substance. Braces may also be used to prevent animals from ingesting test substance, but complete fixation is not desirable.

4. Administration period

It is desirable to administer the substance for 21 consecutive days, with 6 hours of exposure per day, 7 days per week.

5. Determining the number of animals, and establishing test groups

(1) Determining the number of animals

(i) Use 5 male and 5 female animals per group in the studies.

(ii) Use an appropriate random sampling method, by body weight differential, etc., for allocating animals to groups. It is essential to ensure that there will ultimately be a sufficient number of animals for evaluation of test results.

(2) Establishing test groups

(i) Establishing test substance administration groups

a. In addition to the control group, establish test substance dosage groups according to at least 3 dosage levels.

b. Establish dosage levels such that symptoms of test substance toxicity will be clear, and so that the NOAEL can be estimated. As regards maximum dosages, set each dose level such that the dosage at which toxic effects not resulting in death and the minimum dose at which no toxic effects can be determined, and so that the relationship between dosages and response can be discerned. Also indicate the grounds for the dosages established.

c. If no toxic effects are confirmed as a result of administration of the maximum dose that is technically possible, or 1,000 mg/kg of body weight/day of the test substance, it is not necessary to conduct studies with higher dosages than that.

(ii) Control group

a. Establish the same conditions for the control group as for the test substance administration groups, except that the test substance is not administered.

b. When a vehicle, etc. is being used for administration of the test substance, administer to the control group the same amount of vehicle as to the dosage group that is being administered the largest amount of vehicle. Establish an additional untreated control group when using a vehicle, etc. regarding which sufficient information on toxicity cannot be obtained.

6. Observation and examination

Conduct the following items (1)-(5).

(1) Observation of general condition

(i) Observe all animals daily to determine their general condition (times at which symptoms of toxicity occur, and their degree), and mortality. In addition, weigh them regularly, and measure the food consumption.

(ii) In general, weigh the animals and measure the food consumption once prior to commencement of administration, and at least once per week following the commencement of administration.

(2) Blood tests

(i) If would be preferable, in principle, to conduct blood tests of all animals, at least once at the end of the study, in the case of rodents, and both prior to and at the end of the study, in the case of non-rodents.

(ii) It would be desirable for animals to fast for 1 night prior to examination.

(iii) Usually, conduct examinations with regard to the following points. In addition to these, other items could be selected and added as appropriate. Make selections with regard to examination items and methods that are widely accepted internationally.

a. Hematological tests

Red blood cells, white blood cells, hemogram (percentage by leukocyte group), platelet count, hemoglobin, hematocrit; in addition to these, reticulocyte count, clotting capability, (prothrombin time, activated partial thromboplastin time) etc.

b. Blood biochemical tests

Serum (plasma) protein, albumin, glucose, bilirubin, urinary nitrogen, creatinine, transaminase (AST (GOT), ALT (GPT)), γ-GTP, alkaline phosphatase, electrolytes (sodium, potassium, chlorine, calcium, inorganic phosphorus, etc.), etc.

(3) Urinalysis

(i) As regards rodents, conduct urinalyses of a fixed number of male and female animals in each group; as regards non-rodents, conduct urinalyses of all animals. Conduct urinalyses at the same time as blood tests.

(ii) Usually, conduct urinalyses with regard to the following points.

Urine volume, pH, protein, sugar, keton bodies, bilirubin, urobilinogen, occult blood, sediment, specific gravity, etc.

(4) Pathological examinations, etc.

(i) Necropsies

a. Conduct gross examinations of all animals, including those that have died during the study, or those that have been sacrificed due to moribundity.

b. Examine the surface of the body.

c. Weigh the major organs of all animals, including the following organs:

Liver, kidneys, adrenal glands, and testicles

(ii) Preservation of organs and tissues

Preserve organs and tissues as stipulated below.

a. Organs and tissues in which lesions visible to the gross or changes in size are noted.

b. Untreated and treated skin.

c. Liver

d. Kidneys

(iii) Histopathological examinations

Among non-rodents, carry out histopathological examinations of all animals. Among rodents, carry out histopathological examinations of the following, for example.

a. All animals in control and maximum dosage groups.

b. All animals that have died or have been sacrificed during the study period.

c. Parts of any animal in which lesions visible to the gross have occurred.

d. The target tissues of all animals.

e. The livers and kidneys of all animals.

In addition to the above, sites in which effects have occurred in maximum dosage groups must be histologically examined.

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

1. Objective

The objective of these studies is to establish safe methods of handling agricultural chemicals when they are used, by obtaining scientific information regarding toxic changes that occur following repeated exposure to inhalation of the test substance, repeated for 90 days, as well as the maximum dosage at which toxic changes are not observed (NOAEL).

2. Test animals

(1) Use young adult mammals (usually rats) of 1 or more species.

(2) Use young adults.

(3) In general, use the same number of male and female animals. Use nulliparous, non-pregnant females.

3. Exposure method

(1) Use inhalation equipment of appropriate capacity. Do not provide food or water during exposure.

(2) Monitor flow rate, actual concentration of the test substance, particle size distribution, temperature, and humidity during exposure. Maintain the uniformity of these conditions.

(3) Particle size (aerodynamic mass median size) of 1-4 μm is preferable, or use the minimum particle diameter that it is possible to conduct.

(4) If the test substance is volatile, be careful that it is not of a concentration that would cause an explosion.

4. Exposure period

The exposure time should be at least 6 hours per day, at least 5 days per week, for a period of at least 90 days.

5. Determining the number of animals, and establishing test groups

(1) Determining the number of animals

(i) Use at least 10 males and 10 females per group.

(ii) Use an appropriate random sampling method, by body weight differential, etc., for allocating animals to groups. Ensure that there is a sufficient number of animals for evaluation of study results.

(2) Establishing test groups

(i) Establishing test substance administration groups

a. In addition to the control group, establish test substance dosage groups according to at least 3 dosage levels.

b. Establish dosage levels such that symptoms of test substance toxicity will be clear, and so that the NOAEL can be estimated. As regards maximum dosages, set each dose level such that the dosage at which toxic effects not resulting in death and the minimum dose at which no toxic effects can be determined, and so that the relationship between dosages and reactions can be discerned. Indicate the grounds for establishment of dosages.

(ii) Control group

a. Establish the same conditions for the control group as for the test substance administration groups, except that the test substance is not administered.

b. When a vehicle, etc. is being used for administration of the test substance, administer to the control group the same amount of vehicle as to the dosage group that is being administered the largest amount of vehicle. Establish an additional untreated control group when using a vehicle, etc. regarding which sufficient information on toxicity cannot be obtained.

6. Observation and examination

Conduct the following items (1)-(5).

(1) Observation of general condition

(i) Observe all animals daily to determine their general condition (times at which symptoms of toxicity occur, and their degree), and mortality. In addition, weigh them regularly, and measure the food consumption.

(ii) In general, weigh the animals and measure the food consumption once prior to commencement of administration, and at least once per week following the commencement of administration.

(2) Blood tests

(i) If would be desireable, in principle, to conduct blood tests of all animals, at least once at the end of the study. However, based on operational considerations, this may be limited to some of the animals (at least 5) in each group.

(ii) It would be desirable for animals to fast for 1 night prior to examination.

(iii) Usually, conduct examinations with regard to the following points. In addition to these, other items could be selected and added as appropriate. Make selections with regard to examination items and methods that are widely accepted internationally.

a. Hematological tests

Red blood cells, white blood cells, hemogram (percentage by leukocyte group), platelet count, hemoglobin,

hematocrit; in addition to these, reticulocyte count, clotting capability (prothrombin time, activated partial thromboplastin time), etc.

b. Blood biochemical tests

Serum (plasma) protein, albumin, glucose, bilirubin, urinary nitrogen, creatinine, transaminase (AST (GOT), ALT (GPT)), γ-GTP, alkaline phosphatase, electrolytes (sodium, potassium, chlorine, calcium, inorganic phosphorus, etc.), etc.

(3) Urinalysis

(i) Conduct urinalyses of a fixed number of male and female animals (at least 5 of each) in each group at the same time as blood tests.

(ii) Usually, conduct urinalyses with regard to the following points.

Urine volume, pH, protein, sugar, keton bodies, bilirubin, urobilinogen, occult blood, sediment, specific gravity, etc.

(4) Ophthalmological examination

Conduct ophthalmological examinations of all animals, insofar as possible (at least those in the high dosage and control groups), prior to administration and at the end of the study. If abnormalities occur, conduct examinations of all animals.

(5) Pathological examinations, etc.

(i) Necropsies

a. After necropsies have been performed, examine the surface of the body, orifices, cranium, pleural cavity, peritoneal cavity, and internal organs.

b. Weigh the major organs of all animals, including the following organs:

Liver, kidneys, adrenal glands, and testicles

(ii) Preservation of organs and tissues

Preserve organs and tissues as stipulated below, so that it will be possible to conduct histopathological examinations as necessary after the study has been completed.

Organs and tissues in which lesions visible to the gross are noted, skin, brain, pituitary gland, thyroid gland (including the parathyroid gland), thymus gland, lungs (including bronchia), nasopharynx, heart, sternum, salivary gland, liver (including gallbladder), spleen, kidneys, adrenal glands, pancreas, gonads, uterus, appendages to genital organs, lymph nodes, muscle, esophagus, stomach, small intestine (duodenum, jejunum, and ileum), large intestine (cecum, colon, and rectum), bladder, lymph nodes, peripheral nerves, spinal cord, eyes, aorta

(iii) Histopathological examinations

Carry out the following histopathological examinations in regard to all of the following animals, etc.

a. All animals in control and maximum dosage groups.

b. All animals that have died or have been sacrificed during the study period.

c. Parts of any animal in which lesions visible to the gross have occurred.

d. The target tissues of all animals.

e. The esophagus, liver, and kidneys of all animals.

In addition to the above, sites in which effects have occurred in maximum dosage groups must be histologically examined.

Repeated oral neurotoxicity studies (2-1-12)

1. Objective

The objective of these studies is to obtain scientific information regarding toxic changes that occur following repeated oral administration of the test substance, as well as the maximum dosage at which toxic changes are not observed (NOAEL).

These studies may be conducted in conjunction with repeated dose toxicity studies, in order to evaluate the relationship between neurotoxicity and general toxicity.

2. Test animals

(1) Use rodents (rats, usually).

(2) Usually, use, as soon as possible after weaning and following acclimatization period, animals that are the same age, 5-6 weeks old.

(3) In general, use the same number of male and female animals. Use nulliparous, non-pregnant females.

3. Administration method

Carry out repeated orally administration, usually mixed with the animals' feed or water. However, gavage administration may be conducted if these methods of administration prove difficult.

4. Administration period

Conduct for 90 days or 1 year, as necessary.

5. Determining the number of animals, and establishing test groups

(1) Detemrining the number of animals

(i) Use 10 male and 10 female animals in each group for the studies, as necessary for detailed observations of symptoms and examination of functions. From among these, select 5 male and 5 female animals in each group as necessary for neurohistopathological examination.

(ii) Use an appropriate random sampling method, by body weight differential, etc., for allocating animals to groups.

(iii) When conducting these studies in conjunction with others, adjust the number of animals as appropriate, based on the objectives of each study. If interim sacrifice is anticipated, establish the additional number of animals required for this reason. It is essential, ultimately, to ensure that there is a sufficient number of animals for evaluation of study results.

(2) Establishing test groups

(i) Establishing test substance administration groups

a. In addition to the control group, establish test substance dosage groups according to at least 3 dosage levels.

b. Establish dosage levels such that symptoms of test substance toxicity will be clear, and so that the NOAEL can be estimated. As regards maximum dosages, set each dose level such that the dosage at which toxic effects not resulting in large numbers of fatalities and the minimum dosage at which no toxic effects can be determined, and so that the relationship between dosages and reactions can be discerned.

c. Refer to the results of previously conducted toxicity studies when establishing dosages. In addition, indicate the grounds for the dosages established.

d. If no toxic effects are confirmed as a result of administration of the maximum dose that is technically possible, or 1,000 mg/kg of body weight/day of the test substance, it is not necessary to conduct studies with higher dosages than that.

(ii) Control group

a. Establish the same conditions for the control group as for the test substance administration groups, except that the test substance is not administered.

b. When a vehicle, etc. is being used for administration of the test substance, administer to the control group the same amount of vehicle as to the dosage group that is being administered the largest amount of vehicle.

c. Establish an additional untreated control group when using a vehicle, etc. regarding which sufficient information on toxicity cannot be obtained.

6. Observation and examination

Conduct the following items (1)-(6).

(1) Observation of general condition

(i) Observe the general condition of all animals carefully every day.

(ii) Observe general condition, and whether or not abnormal behavior or deaths occur.

(iii) Body weight and food consumption (When the test substance is administered in drinking water, also measure the water consumption. Same below.)

a. For 90-day studies, weigh the animals and measure the amount of feed they ingest once prior to commencement of administration, and at least once per week following the commencement of administration.

b. For 1-year studies, weigh the animals and measure the food consumption once prior to commencement of administration, and at least once per week for 3 months following the commencement of administration, and at least once every 4 weeks thereafter.

c. Compute the amount of test substance ingested.

(2) Detailed observation of condition

(i) Observe 10 males and 10 females from each group studied.

(ii) The frequency of observations is indicated in the table below.

(iii) If repeated dose groups are used, examine animals upon completion of the administration period.

(iv) Observe the animals both in their cages and on the observation stand. Observe the animals carefully, in accordance with a standards observation procedure in which observation items, as well as the definitions of these items, and the scoring criteria and order of observation, have been established sufficiently for ascertaining changes in general condition.

(v) In general, conduct examinations in regard to the following items:

Appearance (skin; fur; changes in eyes, eyeballs, and mucous membranes; presence or absence of discharges; etc.), body position and posture (hunchback posture, etc.), autonomic nervous system function (lacrimation, piloerection, pupil diameter, respiration, excretion, etc.), mortor coordination, ambulatory abnormalities, animal's reactions to being handled and to environmental stimulation, nervous system (tremor, convulsion, muscular contractions, etc.), changes in exploratory behavior, ordinary behavior (changes in grooming, headshaking, gyration, etc.), abnormal behavior (autophagia, backward motion, abnormal vocalization, etc.), aggression, etc.

(3) Functional examination

(i) Observe 10 males and 10 females from each group studied.

(ii) The frequency of examinations is indicated in the table below.

(iii) Conduct examination of rodents near the end of administration, insofar as possible.

(iv) In general, conduct examinations in regard to the following items:

Sensorimotor reactions to stimuli (including auditory, visual, and proprioceptor stimuli), grip strength, and amount of spontaneous motor activity(measure using an automatic recording apparatus).

(v) If it is suspected, on the basis of other toxicity studies, that the test substance is a neurotoxin, conduct studies and careful examinations regarding the appropriate sensory mechanisms, motor function, and learning/memory relating to the suspected neurotoxicity.

(4) Ophthalmological examination

Conduct ophthalmological examinations of, at least, animals in the high dosage and control groups, prior to administration and at the end of the study. If abnormality is observed, ascribable to the test substance occur, conduct examinations of all animals.

(5) Histopathological examinations

(i) Examine 5 males and 5 females from each group studied.

(ii) Examine specific animals in which abnormalities were observed in observations of symptoms and in functional examinations.

(iii) Fix the tissue by means of perfusion fixation, or other appropriate method. Record all gross observations of physiological changes.

(iv) In general, embed tissue sample in paraffin, and use a staining agent, such as hematoxylin and eosin stain. However, if nerve damage in the peripheral nervous system is observed or suspected, adjust and examine peripheral nerve tissues embedded in resin.

(v) Conduct additional examinations and special staining, as appropriate, based on symptomatic observations.

(vi) Evaluate neurohistopathological findings by relating them to other toxicity study results and behavioral effects.

(vii) Conduct phased examinations of representative sections from the central and peripheral nervous systems.

(viii) First of all, compare sections from the maximum dosage group and the control group. If no neurohistopathological changes are observed in the maximum dosage group, no further examinations are necessary. If changes are observed in the maximum dosage group, examine sections from the median and minimum dosage groups, in that order.

(ix)In general, examine the following tissues:

The central cerebrum, including the prosencephalon and the hippocampus; the mesencephalon, cerebellum, pons, and medulla oblongata; the eyeball, including the optic nerve and retina; the cervical and lumbar enlargements of the spinal cord; the spinal ganglion, the ventral and dorsal roots of nerve fibers, the proximal sciatic nerve, the proximal tibial nerve (in the knee area), the gastrocnemius bifurcation of the tibial nerve, and the skeletal muscles (especially the gastrocnemius).

(x) The sections from the spinal cord and peripheral nerves should include both horizontal and vertical sections.

(xi) Preserve organs and tissues, so that it will be possible to conduct histopathological examinations as necessary after the study has been completed.

Table: Frequency of detailed observation of symptoms and functional studies

Type of Study	Relevant animals	90-day studies	1-year studies
Observation of general condition	All animals	Every day	Every day
Detailed observation of condition	Animals selected for detailed observation	(1) Prior to commencement of administration (2) Once during the 1st or 2nd week of administration (3) Each month after commencement of administration	(1) Prior to commencement of administration (2) Once, 1 month after commencement of administration (3) Every 3 months after commencement of administration
Functional examination	Animals selected for functional examination	(1) Prior to commencement of administration (2) Once during the 1st or 2nd week of administration (3) Each month after commencement of administration	(1) Prior to commencement of administration (2) Once, 1 month after commencement of administration (3) Every 3 months after commencement of administration

28-day repeated dose delayed neurotoxicity studies (2-1-13)

1. Objective

The objective of these studies is to obtain information regarding the details of toxic changes that occur following repeated dose of the test substance over a 28-day period, and the maximum dosage at which toxic changes are not observed (NOAEL), in order to provide additional information regarding delayed neurotoxicity that has been confirmed or is suspected on the basis of acute delayed neurotoxicity studies.

2. Test animals

(1) Use female chickens of ordinary varieties and strains.

(2) It is desirable to use young adult chickens of standard size, 8-12 months old.

(3) Use healthy animals that have not had viral diseases or drug treatments that would affect actual study results, nor any ambulatory abnormalities.

3. Administration method

(1) Carry out successive forcible oral administration, by means of stomach tube, gelatin capsule, or an equivalent method.

(2) If the test substance is a liquid, administer it as liquid concentrate, or after dissolving in an appropriate vehicle. If the test substance is a solid, administer it in solution, insofar as possible.

4. Administration and observation periods

(i) The administration period is 28 days.

(ii) Conduct observations for 14 days following the end of administration.

5. Determining the number of animals, and establishing test groups

(1) Determining the number of animals

Use a number of animals in both the administration and control groups such that the 6 birds needed for biochemical tests, as well as 6 birds that can survive until the end of the studies and can be used in histopathological studies, will be available.

(2) Establishing test groups

(i) Establishing test substance administration groups

a. Establish test substance dosage groups according to at least 3 dosage levels.

b. Establish dosage levels such that the toxic effect of the test substance, and insofar as possible its delayed neurotoxicity, will be clear, and so that the animals do not die or show conspicuous signs of suffering. Set the dosage at which no toxic effects can be determined as the minimum dosage. Set other dosage levels such that the relationship between dosages and reactions can be discerned.

c. Refer to the results of acute delayed neurotoxicity studies, and other toxicity studies, when establishing dosages. In addition, indicate the grounds for the dosages established.

d. If no toxic effects are confirmed as a result of administration of the maximum dose that is technically possible, or 1,000 mg/kg of body weight/day of the test substance, it is not necessary to conduct studies with higher dosages than that.

(ii) Control group

a. Establish the same conditions for the control group as for the test substance administration groups, except that the test substance is not administered.

b. When a vehicle, etc. is being used for administration of the test substance, administer to the control group the same amount of vehicle as to the dosage group that is being administered the largest amount of vehicle. Establish an additional untreated control group when using a vehicle, etc. regarding which sufficient information on toxicity cannot be obtained.

6. Note regarding care

Keep the animals in a cage or pen that is large enough to allow the animals to walk around freely, and readily permit observation of their ambulatory status.

7. Observation and examination

Conduct the following items (1)-(3).

(1) Observation of general condition

(i) Commence observation of all animals immediately after administration. Carefully observe them at least once per day during the administration period, and for 14 days after the end of the administration period, or until planned sacrifice.

(ii) Keep records of all symptoms of toxicity, including their type, time of occurrence, degree, and duration. Evaluate ataxia on the basis of criteria comprising at least 4 levels.

(iii) Animals used in pathological examinations should be brought out of their cages at least twice per week. Conduct forced movement at fixed times, in order to be able to observe even slight toxic effects.

(iv) Conduct gross pathological examinations of moribund animals after removing and sacrificing them.

(v) Weigh all animals once prior to administration and once 1 week after administration.

(2) Biochemical examinations

(i) Sacrifice 6 birds each, chosen at random, from the administration and control groups, 72 hours after administration of the test substance. Harvest their brains and lumbar spinal cords, and measure the neuropathy target esterase (NTE, also called neurotoxic esterase) activity.

Usually, sacrifice 3 birds each from the administration and control groups 24 hours and 48 hours after administration, and harvest their brains and lumbar spinal cords.

If, based on the results of acute delayed neurotoxicity studies or other study results, another interval is considered more appropriate for detecting the capability of inducing delayed neurotoxicity, it would be desirable to sacrifice 3 birds for a second time, and harvest their brains and lumbar spinal cords.

(ii) Measuring the acetylcholine esterase activity in the relevant organs (brain and cerebrospinal system) of the same animals in which NTE activity was measured would be helpful in evaluation.

(3) Pathological examinations

(i) Conduct gross pathological examinations of all animals sacrificed, either as planned or as circumstances require, including observation of the appearance of the brain and spinal cord.

(ii) Conduct studies of nerve tissue harvested from at least 6 surviving birds from each group when the study is complete.

(iii) Fix the tissue by means of perfusion fixation, or other appropriate method.

a. Include sections from the cerebellum (central longitudinal plane), medulla oblongata, spinal cord, and peripheral nerves.

b. Collect spinal cord sections from the upper cervical, mesothorax, and lumbo-sacral areas.

c. Collect proximal, distal, and bifurcating sections of the tibial nerve, and also harvest the sciatic nerve.

(iv) Stain sections of the myelin sheaths and the axons according to an appropriate procedure.

1-year repeated oral toxicity studies (2-1-14)

1. Objective

The objective of these studies is to obtain scientific information regarding toxic changes that occur following long-term oral administration of the test substance, as well as the maximum dosage at which clear signs of toxic changes are not observed (NOAEL: NOAEL, no observed adverse effect level).

2. Test animals

(1) Conduct studies with 1 species of rodent (usually, rats) and 1 species of non-rodent (usually, dogs).

(2) As regards rodents, use as soon as possible after weaning and following acclimatization period (usually, 5-6 weeks old); as regards non-rodents (dogs), use animals that are 4-6 months old.

(3) In principle, use the same number of male and female animals. Use nulliparous and non-pregnant females.

3. Administration method

Carry out repeated oral administration, usually mixed with the animals' feed or water. However, gavage administration may be conducted if these methods of administration prove difficult.

4. Administration period

Repeated for at least 1 year.

5. Determining the number of animals, and establishing test groups

(1) Determining the number of animals

(i) As regards rodents, use at least 20 males and 20 females per group; as regards non-rodents, use at least 4 males and 4 females per group.

(ii) Use an appropriate random sampling method, by body weight differential, etc., for allocating animals to groups. If interim sacrifice is anticipated, establish the additional number of animals required for this reason.

(iii) It is essential to ensure that there is a sufficient number of animals for evaluation of study results.

(2) Establishing test groups

(i) Establishing test substance administration groups

a. In addition to the control group, establish test substance dosage groups according to at least 3 dosage levels.

b. Establish dosage levels such that symptoms of test substance toxicity will be clear, and so that the NOAEL can be estimated. As regards maximum dosages, set each dose level such that the dosage at which toxic effects not resulting in death and the minimum dose at which no toxic effects can be determined, and so that the relationship between dosages and response can be discerned.

c. Refer to the results of 90-day repeated dose oral toxicity studies in establishing dosages. Indicate the grounds for establishment of dosages.

d. If no toxic effects are confirmed as a result of administration of the maximum dose that is technically possible, or 1,000 mg/kg of body weight/day of the test substance, it is not necessary to conduct studies with higher dosages than that.

(ii) Control group

a. Establish the same conditions for the control group as for the test substance administration groups, except that

the test substance is not administered.

b. When a vehicle, etc. is being used for administration of the test substance, administer to the control group the same amount of vehicle as to the dosage group that is being administered the largest amount of vehicle.

c. Establish an additional untreated control group when using a vehicle, etc. regarding which sufficient information on toxicity cannot be obtained.

6. Observation and examination

Conduct the following items (1)-(5).

(1) Observation of general condition

(i) Observe daily the general condition of all animals.

(ii) Weigh the animals regularly, and measure the food consumption. (When the test substance is administered in drinking water, also measure the water consumption. Same below.)

(iii) In general, weigh the animals and measure the food consumption, once prior to commencement of administration, and at least once per week until 3 months after the commencement of administration. Thereafter, weigh them at least once every 4 weeks. Also compute the amount of test substance ingested.

(2) Blood tests

(i) Conduct tests 6 months following commencement of administration and at the end of the study, in the case of rodents, and prior to commencement of administration, 6 months following commencement of administration and at the end of the study, in the case of non-rodents. In principle, test all animals, but in the case of rodents, the examinations may be limited to a part of each group (at least 10 males and 10 females), for operational reasons.

(ii) Except in the case of mice, it would be desirable for animals to fast for 1 night prior to examination.

(iii) Usually, conduct examinations with regard to the following points. In addition to these, other items should be selected and added as appropriate. Make selections with regard to examination items and methods that are widely accepted internationally.

a. Hematological tests

Red blood cells, white blood cells, hemogram (percentage by leukocyte group), platelet count, hemoglobin, hematocrit; in addition to these, reticulocyte count, clotting capability (prothrombin time, activated partial thromboplastin time), etc.

b. Blood biochemical tests

Serum (plasma) protein, albumin, A/G ratio, glucose, cholesterol, triglyceride, bilirubin, urinary nitrogen, creatinine, transaminase (AST (GOT), ALT (GPT)), γ-GTP, alkaline phosphatase, electrolytes (sodium, potassium, chlorine, calcium, inorganic phosphorus, etc.), etc.

(3) Urinalysis

(i) As regards rodents, conduct urinalyses of a fixed number (10 or more) of male and female animals in each group; as regards non-rodents, conduct urinalyses of all animals. Conduct urinalyses at the same time as blood tests.

(ii) It is best to conduct urinalysis of the same animals used for blood tests.

(iii) Usually, conduct urinalyses with regard to the following points.

Urine volume, pH, protein, sugar, keton bodies, bilirubin, urobilinogen, occult blood, sediment, specific gravity, etc.

(4) Ophthalmological examination

Conduct ophthalmological examinations of all animals among non-rodents, and among at least the high dosage and control groups among rodents, prior to administration and at the end of the study. If abnomarlity is observed ,that are ascribable to the test substance, conduct examinations of all animals.

(5) Pathological examination, etc.

(i) Promptly conduct necropsies, as well as gross observations, of organs and tissues, and histopathological examinations, in cases of deaths during the administration period. Strive to determine the cause of death, and the degree of toxic changes at the time of death.

(ii) Promptly sacrifice and dissect animals that become moribund during the study period; conduct observations

and examinations as in (i) above. Determine the cause of moribundity, and the degree of toxic changes at the time the animals became moribund.

(iii) Sacrifice and dissect all surviving animals at the end of the administration period, after having collected blood and urine for the various tests. Conduct gross observations of organs and tissues. Usually, weigh the following organs. Except in the case of mice, it would be desirable for animals to fast for 1 night prior to examination.

Liver, kidneys, adrenal glands, testes, ovaries, spleen, heart, brain, prostate gland(NOTE), thyroid gland, parathyroid gland(NOTE), and pituitary gland(NOTE)

NOTE: Examine the prostate, parathyroid, and pituitary glands of non-rodents only.

(iv) Conduct histopathological examinations of all animals among non-rodents, and at least of those in the high dosage and control groups among rodents.

(v) Usually, conduct pathological examinations of the following organs and tissues, and add others as appropriate upon gross examination.

Skin, mammary glands, lymph nodes, (cervical and mesenteric lymph nodes, etc.), aorta, salivary glands, bones and marrow (sternum and femur), thymus gland, trachea, lungs and bronchia, heart, thyroid and parathyroid glands, esophagus, stomach, small intestine (duodenum, jejunum, and ileum), large intestine (cecum, colon, and rectum), liver (and gallbladder), pancreas, spleen, kidneys, adrenal glands, bladder, seminal vesicle and coagulating gland, prostate, testes, epididymis, ovaries, uterus, vagina, brain, pituitary gland, sciatic nerve, skeletal muscle, spinal cord (cervical, thoracic, and lumbar areas), eyeballs and appendages, and other organs and tissues in which changes can be confirmed with the gross.

(vi) If, in dosage groups of animals other than rodents, changes due to administration of the test substance are observed in the relevant organs and tissues with the gross, during 90-day repeated dose oral toxicity studies or if it is deemed necessary on the basis of findings in high dosage groups, conduct histopathological examinations of all animals in those groups.

(vii) Among rodents, histopathological examination of all animals would be helpful in evaluations.

(viii) Even after the completion of studies, preserve organs and tissues so that histopathological examinations can be conducted if necessary.

Carcinogenicity studies (2-1-15)

1. Objective

The objective of these studies is to obtain scientific findings regarding whether or not repeated oral administration of the test substance is carcinogenic.

2. Test animals

(1) Use at least 2 species of rodent (usually, rats and mice).

(2) Usually, use, as soon as possible after weaning and following acclimatization period, animals that are the same age, 5-6 weeks old. As regards selection of varieties and strains, those that are known to have such characteristics as resistance to infectious diseases, long life, and sensitivity to known carcinogens are widely used as test animals. In particular, select strains regarding which there is accumulated evidence regarding incidence of spontaneous tumors.

(3) In general, use the same number of male and female animals. Use nulliparous, non-pregnant females.

3. Administration method

Carry out repeated oral administration, usually mixed with the animals' feed or water. However, gavage administration may be conducted if these methods of administration prove difficult.

4. Administration period

(1) Establish the test substance administration period necessary for achievement of study objectives, taking into account the average life expectancy of the varieties and strains of animals used.

(2) Usually, the administration period should be 24-30 months for rats, and 18-24 months for mice.

5. Determining the number of animals, and establishing test groups

(1) Determining the number of animals

(i) Use 50 male and 50 female animals in each group.

(ii) Use an appropriate random sampling method, by body weight differential, etc., for allocating animals to groups.

(iii)It is unacceptable to lose 10% or more of any group, due to cannibalism or other causes related to animal care.

(iv) In principle, it is unacceptable for the survival rate of rats (at 24 months after the commencement of administration) or mice (at 18 months after the commencement of administration) to fall below 25%. If interim sacrifice is anticipated, establish the additional number of animals required for this reason.

(2) Establishing test groups

(i) Test substance administration groups

a. In addition to the control group, establish test substance dosage groups according to at least 3 dosage levels.

b. Establish groups according to the dosage levels listed below, such that the relationship between dosage and reaction will be clear. Refer to the results of 90-day repeated oral toxicity studies in establishing dosages. Indicate the grounds for establishment of dosages.

(ii) Establishing dosages

a. Maximum dosage

Select as the maximum a dosage at which several toxic affects are confirmed, but at which the death rate from causes other than tumors is not significantly higher than that of the control group.

If no toxic effects are confirmed as a result of administration of the maximum dose that is technically possible, or 1,000 mg/kg of body weight/day of the test substance, it is not necessary to conduct studies with higher dosages than that.

b.Minimum dosage

Usually, the minimum dose must not be less than 10% of the maximum dose.

c. Intermediate dosage

It is desirable to establish the geometrical mean of the maximum and minimum dosages as the intermediate dosage. Usually, the common ratio between groups is a value from 2 to 3.

d.Other

It is not necessary to determine NOAEL of the test substance on the basis of these studies, but if carcinogenicity, etc., is noted, determine its mechanism through additional studies, etc., and determine the NOAEL of the test substance with respect to carcinogenicity, according to appropriate parameters.

(iii) Control group

a. Establish the same conditions for the control group as for the test substance administration group, except that the test substance is not administered.

b.When a vehicle, etc. is being used for administration of the test substance, administer to the control group the same amount of vehicle as to the dosage group that is being administered the largest amount of vehicle. Establish an additional untreated control group when using a vehicle, etc. regarding which sufficient information on toxicity cannot be obtained.

6. Observation and examination

Conduct the following items (1)-(3).

(1) Observation of general condition

(i) In general, observe daily the general condition of all animals. In addition, weigh them regularly, and measure the food consumption. (When the test substance is administered in drinking water, also measure the water consumption. Same below.)

(ii) In general, weigh the animals and measure the food consumption once prior to commencement of administration, and at least once per week for 3 months following the commencement of administration, and at

least once every 4 weeks thereafter.

(2) Blood tests

Adjust blood samples of animals that have died or become moribund during the study period, or when sacrificing survivors at the end of the study period. Adjust smears of examples in which hematopoietic tumors are anticipated, due to tumefaction, etc. of the thymus gland, lymph nodes, liver, or spleen.

(3) Pathological examination, etc.

(i) Promptly conduct necropsies, as well as gross observations, of organs and tissues, and histopathological examinations, in cases of fatality during the study period. It is necessary to add findings on all kinds of changes (hyperplasia, precancerous lesion, etc.), up to and including tumors, in records of tumorous lesions (same as regards (ii) and (iii) below).

(ii) Usually, conduct pathological examinations of the following organs and tissues, and add others as appropriate upon gross examination.

Skin, mammary glands, lymph nodes, (cervical and mesenteric lymph nodes, etc.), aorta, salivary glands, bones and marrow (sternum and femur), thymus gland, trachea, lungs and bronchia, heart, thyroid and parathyroid glands, esophagus, stomach, small intestine (duodenum, jejunum, and ileum), large intestine (cecum, colon, and rectum), liver (and gallbladder), pancreas, spleen, kidneys, adrenal glands, bladder, seminal vesicle and coagulating gland, prostate, testes, epididymis, ovaries, uterus, vagina, brain, pituitary gland, sciatic nerve, skeletal muscle, spinal cord (cervical, thoracic, and lumbar areas), eyeballs and appendages, nasal cavity, and other organs and tissues in which changes can be confirmed with the gross.

(iii) Promptly sacrifice and dissect animals that become moribund during the study period; conduct observations and examinations as in (i) above.

(iv) At the end of the study period, promptly sacrifice and dissect all surviving animals, and examine organs and tissues with the gross. Conduct histopathological examinations, as in (i) above, of all animals in the control and maximum dosage groups. However, if there are organs or tissues regarding which there is a difference in rate of tumorigenesis between the maximum dosage and control groups, conduct histopathological examinations of the relevant organs and tissues of all other animals in the other dosage groups.

It would be helpful in evaluations to conduct histopathological examinations of all animals.

(v) Even after the completion of studies, preserve organs and tissues so that histopathological examinations can be conducted if necessary.

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

1. Objective

These studies are conducted in order to detect adverse effects that occur following long-term repeated dose of the test substance, and their objective is to obtain scientific information regarding the 1-year repeated dose oral toxicity and carcinogenicity of the test substance simultaneously.

2. Test animals

(1) Conduct the studies using 1 species of rodent (usually, rats). Use, as soon as possible weaning and following acclimatization period, animals that are same age (5-6 weeks old, usually).

(2) As regards selection of varieties and strains, those that are known to have such characteristics as resistance to infectious diseases, long life, and sensitivity to known carcinogens are widely used as test animals. In particular, select strains regarding which there is accumulated evidence regarding incidence of spontaneous tumors.

(3) In general, use the same number of male and female animals. Use females that have never given birth and are not pregnant.

3. Administration method

Carry out repeated oral administration, usually mixed with the animals' feed or water. However, forcible oral

administration may be conducted if these methods of administration prove difficult.

4. Administration period

(1) Establish the test substance administration period necessary for achievement of study objectives, taking into account the average life expectancy of the varieties and strains of animals used.

(2) Usually, the administration period should be 24-30 months for rats, and 18-24 months for mice.

(3) For the satellite group and its control group, the period for detecting 1-year repeated oral toxicity should be at least 1 year, in general.

5. Determining the number of animals, and establishing test groups

(1) Determining the number of animals

(i) Use 50 male and 50 female animals in each group.

(ii) Use an appropriate random sampling method, by body weight differential, etc., for allocating animals to groups.

(iii) It is unacceptable to lose 10% or more of any group, due to cannibalism or other causes related to animal care.

(iv) In principle, it is unacceptable for the survival rate of rats (at 24 months after the commencement of administration) or mice (at 18 months after the commencement of administration) to fall below 25%. If interim sacrifice is anticipated, establish the additional number of animals required for this reason.

(v) Regarding the satellite groups

a. The number of animals established for detecting 1-year repeated dose oral toxicity should be at least 10 males and 10 females per group. However, 20 of each should be used in the maximum dosage group.

b.Use an appropriate random sampling method, by body weight differential, etc., for allocating animals to groups.

c. If interim sacrifice is anticipated, establish the additional number of animals required for this reason.

d.It is essential to be able to ensure that the number of animals is sufficient for evaluating study results.

(2) Establishing test groups

(i) Dosage levels for detecting carcinogenicity

In addition to the control group, establish test substance dosage groups according to at least 3 dosage levels. In addition to these, establish satellite groups and a control group for 1-year repeated oral toxicity studies.

Establish groups according to the dosage levels listed below, such that the relationship between dosage and reaction will be clear.

Refer to the results of 90-day repeated dose oral toxicity studies in establishing dosages. Indicate the grounds for establishment of dosages.

a. Maximum dosage

Select as the maximum a dosage at which several toxic affects are confirmed, but at which the death rate from causes other than tumors is not significantly higher than that of the control group.

If no toxic effects are confirmed as a result of administration of the maximum dose that is technically possible, or 1,000 mg/kg of body weight/day of the test substance, it is not necessary to conduct studies with higher dosages than that.

b.Minimum dosage

Usually, the minimum dose must not be less than 10% of the maximum dose.

c. Intermediate dosage

It is best to establish the geometrical mean of the maximum and minimum dosages as the intermediate dosage. Usually, the common ratio between groups is 2 to 3.

d.Other

It is not necessary to determine NOAEL of the test substance on the basis of these studies, but if carcinogenicity, etc., is noted, determine its mechanism through additional studies, etc., and determine the NOAEL of the test substance with respect to carcinogenicity, according to appropriate parameters.

(ii) Dosage levels for detecting 1-year repeated dose oral toxicity

a. In addition to the control group, establish test substance dosage groups according to at least 3 dosage levels.

b.Establish dosage levels such that symptoms of test substance toxicity will be clear, and so that the NOAEL can be estimated. As regards maximum dosages, set each dose level such that the dosage at which toxic effects not resulting in death and the minimum dose at which no toxic effects can be determined, and so that the relationship between dosages and reactions can be discerned.

c. Refer to the results of 90-day repeated dose oral toxicity studies in establishing dosages. Indicate the grounds for establishment of dosages.

d.If no toxic effects are confirmed as a result of administration of the maximum dose that is technically possible, or 1,000 mg/kg of body weight/day of the test substance, it is not necessary to conduct studies with higher dosages than that.

(iii) Control group

a. Establish the same conditions for the control group as for the test substance administration groups, except that the test substance is not administered.

b.When a vehicle, etc. is being used for administration of the test substance, administer to the control group the same amount of vehicle as to the dosage group that is being administered the largest amount of vehicle.

c. Establish an additional untreated control group when using a vehicle, etc. regarding which sufficient information on toxicity cannot be obtained.

6. Observation and examination

Conduct the following items (1)-(5).

(1) Observation of general condition

(i) Observe daily the general condition of all animals. Weigh them regularly, and measure the food consumption. (When the test substance is administered in drinking water, also measure the water consumption. Same below.)

(ii) In general, weigh the animals and measure the food consumption once prior to commencement of administration, and at least once per week until 3 months after the commencement of administration. Thereafter, weigh them at least once every 4 weeks. Also compute the amount of test substance ingested.

(2) Blood tests

(i) In the dosage groups for detection of carcinogenicity, adjust blood samples of animals that have died or become moribund during the study period, or when sacrificing survivors at the end of the study period. Adjust smears of examples in which hematopoietic tumors are anticipated, due to tumefaction, etc. of the thymus gland, lymph nodes, liver, or spleen.

(ii) In the satellite groups for detection of 1-year repeated oral toxicity, collect blood samples from 10 males and 10 females in each group 6 months after the commencement of administration and at the end of the study (at 12 months). Conduct hematological and blood biochemical tests.

(iii) Except in the case of mice, it would be desirable for animals to fast for 1 night prior to examination.

(iv) Usually, conduct examinations with regard to the following points. In addition to these, other items should be selected and added as appropriate. Make selections with regard to examination items and methods that are widely accepted internationally.

a. Hematological tests

Red blood cells, white blood cells, hemogram (percentage by leukocyte group), platelet count, hemoglobin, hematocrit; in addition to these, reticulocyte count, clotting capability (prothrombin time, activated partial thromboplastin time), etc.

b.Blood biochemical tests

Serum (plasma) protein, albumin, A/G ratio, glucose, cholesterol, triglyceride, bilirubin, urinary nitrogen, creatinine, transaminase (AST (GOT), ALT (GPT)), γ-GTP, alkaline phosphatase, electrolytes (sodium, potassium, chlorine, calcium, inorganic phosphorus, etc.), etc.

(3) Urinalysis

(i) Conduct urinalyses of a fixed number (10 or more) of male and female animals in each satellite group for detection of 1-year repeated oral toxicity; as regards non-rodents, conduct urinalyses of all animals. Conduct urinalyses at the same time as blood tests.

(ii) It is desirable to conduct urinalysis of the same animals used for blood tests.

(iii) Usually, conduct urinalyses with regard to the following points.

Urine volume, pH, protein, sugar, keton bodies, bilirubin, urobilinogen, occult blood, sediment, specific gravity, etc.

(4) Ophthalmological examination

Conduct ophthalmological examinations of at least the high dosage and control groups among satellite groups for detection of 1-year repeated oral toxicity, prior to administration and at the end of the study. If abnormality is observed that are ascribable to the test substance, conduct examinations of all animals.

(5) Pathological examination, etc.

(i) Promptly conduct necropsies, as well as gross observations, of organs and tissues, and histopathological examinations, in cases of deaths during the administration period. Strive to determine the cause of death, and the degree of toxic changes at the time of death.

It is necessary to add findings on all kinds of changes (hyperplasia, precancerous lesion, etc.), up to and including tumors, in records of tumorous lesions (same as regards (ii) and (iii) below).

(ii) Usually, conduct pathological examinations of the following organs and tissues, and add others as appropriate upon gross examination.

Skin, mammary glands, lymph nodes, (cervical and mesenteric lymph nodes, etc.), aorta, salivary glands, bones and marrow (sternum and femur), thymus gland, trachea, lungs and bronchia, heart, thyroid and parathyroid glands, esophagus, stomach, small intestine (duodenum, jejunum, and ileum), large intestine (cecum, colon, and rectum), liver (and gallbladder), pancreas, spleen, kidneys, adrenal glands, bladder, seminal vesicle and coagulating gland, prostate, testes, epididymis, ovaries, uterus, vagina, brain, pituitary gland, sciatic nerve, skeletal muscle, spinal cord (cervical, thoracic, and lumbar areas), eyeballs and appendages, nasal cavity, and other organs and tissues in which changes can be confirmed with the gross.

(iii) Promptly sacrifice and dissect animals that become moribund during the study period; conduct observations and examinations as in (i) above. Strive to clarify the cause of moribundity, and the degree of toxic changes at the time the animals became moribund.

(iv) Sacrifice and dissect all surviving animals in the dosage groups for detection of carcinogenicity and satellite groups for detection of 1-year repeated oral toxicity at the end of the administration period, and conduct gross examinations of organs and tissues. Weigh the organs of all animals in the satellite groups. Usually, weigh the following organs. Except in the case of mice, it would be desirable for animals to fast for 1 night prior to examination.

Liver, kidneys, adrenal glands, testes, ovaries, spleen, heart, brain

(v) Conduct histopathological examinations, as in (i) above, of all animals in the control and maximum dosage groups. However, conduct histopathological examinations of organs or tissues of all other animals regarding which a difference in rate of tumorigenesis between the maximum dosage and control groups is noted, of the relevant organs and tissues in 90-day repeated oral toxicity studies, of sites in which lesions are noted with the gross in the course of these studies, and organs and tissues regarding which such examinations are deemed necessary on the basis of findings in the high dosage groups.

It would also be helpful in evaluation to conduct histopathological examinations of all animals in circumstances other than those noted above.

(vi) Even after the completion of studies, preserve organs and tissues so that histopathological examinations can be conducted in the future if necessary.

Reproductive toxicity studies (2-1-17)

1. Objective

The objective of these studies is to obtain scientific information regarding the effect of administering the test substance to two generations (first generation (P) and second generation (F1)) of animals on their reproductive functions, such as

estrous cycle, coitus, conception, parturition, and lactation, and on their offspring.

2. Test animals

(1) Use at least 1 species of rodent (usually, rats).

(2) When selecting strains of test animals, avoid those with a low fertility. Select strains that were bred for use in general toxicity and reproductive toxicity.

3. Administration method

(1) Carry out repeated dose oral administration, usually mixed with the animals' feed or water. However, gavage administration may be conducted if these methods of administration prove difficult.

(2) Computes dosages weekly based on the body weight of each individual. Dosages for pregnant females may be computed on the basis of body weight on gestation days 0 and 6.

4. Administration period

The test substance administration period should be as follows.

(1) First generation (P)

Commence administration from the time animals are 5-9 weeks old, after acclimating for 5 days, and continue for at least 10 weeks, until mating. Thereafter, continue administering to males at least until they have stopped mating, and to females until the weaning of F1 offspring.

(2) Second generation (F1)

Commence administration at the time of weaning until mating. Thereafter, continue administering to males at least until they have stopped mating, and to females until the weaning of F2 offspring.

5. Determining the number of animals, and establishing test groups

(1) Determining the number of animals

Use an equal number of male and female animals in the studies sufficient for obtaining at least 20 gravid animals per group.

(2) Establishing test groups

(i) Establishing test substance administration groups

a. Establish test substance dosage groups according to at least 3 dosage levels.

b. Establish dosage levels such that symptoms of test substance toxicity will be clear, and so that the NOAEL can be estimated. Set the maximum dosage such that the dosage at which toxic effects not resulting in death, such as suppression of weight gain, are observed in parents or offspring, and the minimum dose at which no toxic effects can be determined in either parents or offspring, and set other dosage levels so that the dose –response relationship can be discerned.

c. If no toxic effects are confirmed as a result of administration of the maximum dose that is technically possible, or 1,000 mg/kg of body weight/day of the test substance, it is not necessary to conduct studies with higher dosages than that.

(ii) Control group

a. Establish the same conditions for the control group as for the test substance administration groups, except that the test substance is not administered.

b. When a vehicle, etc. is being used for administration of the test substance, administer to the control group the same amount of vehicle as to the dosage group that is being administered the largest amount of vehicle. Establish an additional untreated control group when using a vehicle, etc. regarding which sufficient information on toxicity cannot be obtained.

6. Mating, adjusting number of animals per litter, and second generation (F1) selection

(1) First generation (P)

(i) Allow male/female pairs from the same dosage groups to cohabit and mate until copulation is confirmed. Limit

the cohabitation period to 2 weeks.

(ii) Check daily whether coitus has occurred, according to the presence or absence of spermatozoa in vaginal smears, or the presence or absence of a vaginal plug. Establish the day on which spermatozoa or vaginal plug are confirmed as gestation day 0.

(iii) Obtaining second litter from the first generation may be considered, as necessary.

(iv) Allow litters to nurse until weaning. In adjusting the number per litter, exclude excess neonates at random, 4 days after birth, until 4 males and 4 females remain in each. If it is impossible to make adjustments to obtain 4 male and 4 female animals per litter, there is no objection to making adjustments such that there are a total of 8 animals (for example, 5 males and 3 females) per litter. Do not adjust litters of less than 8 offspring.

(v) When weaning F1, make selections so that males and females that each mate with 1 or 2 partners are, within any given group, from as many mothers as possible.

(2) Second generation (F1)

Allow mating and adjust the number of animals per litter in the same way as in the first generation (P). Prevent mating between members of the same litter.

7. Observation and examination

Conduct the following items (1)-(3).

(1) Parent animals

(i) General condition

a. Observe daily the general condition of P animals, as well as F1 animals used for breeding. During reproductive period, examine females in regard to pregnancy and parturition.

b. Observation of general condition should include signs of excitation, seizure, sedation, and ambulatory irregularity, in addition to survival/fatality and outward appearance. Observations in regard to pregnancy and parturition should include miscarriage, premature birth, and delayed birth.

(ii) Body weight and food consumption

a. In general, observe daily the general condition of all P animals, as well as F1 animals used for breeding. In addition, weigh them regularly, and measure the food consumption. (When the test substance is administered in drinking water, also measure the water consumption. Same below.) In general, the animals should be weighed, and the food consumption measured, on the day administration commences, and at least once per week thereafter.

b. Weigh females during the reproductive period, on gestation days 0, 7, 14, and 21, and on nursing days 0, 7, 14, and 21.

c. Also compute the amount of test substance ingested.

(iii) Observations as to sexual maturation

Observe the development of the external sexual organs of the F1 animals used for mating.

(iv) Estrous cycle

Observe the estrous cycles of P females, as well as F1 females used for mating, at least 2 weeks prior to mating. If necessary, also observe the estrous cycles of 3rd generation (F2) females.

(v) Gestation, parturition, and nursing

a. Compute the following values, based on the number of females engaging in copulation, the number of pregnant females (and the number of males that fertilize females), the number of delivered dams, and the number of offspring weaned.

Copulation rate	< (Number of animals which copulated / number of animals used for mating) x 100 >
Conception rate	< (Number of animals pregnant / number of females which copulated) x 100 >
Birth rate	< (Number of females delivering live offspring / number of pregnant females) x 100 >
Weaning rate	< (Number of surviving offspring at weaning / number of offspring, adjusted 4 days after birth) x 100 >

b. Study causal factors as regards females that fail to copulate successfully.

(vi) Sperm tests

At the time of sacrifice, determine the number, motility, and morphology of spermatids inside the testes and epididymis of P males and F1 males used for mating.

(2) Offspring

(i) Immediately after birth, note the number of offspring of each female, as well as their weight, the number of stillborn offspring, the number of live offspring, and whether or not any of them exhibit external abnormalities. If necessary, measure anogenital distance (AGD).

(ii) Note the number of live offspring on the 4th, 7th, 14th, and 21st days after birth. Compute survival rates and weigh each individual.

(iii) In addition observe the general condition of the offspring.

(3) Pathological examinations

(i) After the offspring of the P animals and the F1 animals used for mating are weaned, promptly sacrifice the F2 young and the F1 animals not used for mating. Dissect them, and conduct gross examinations, with special reference to the organs in the reproductive systems.

(ii) Determine the number of implantations in the uteruses of females used for mating. Dissect individuals that die during the study period promptly, to determine the cause. Also promptly sacrifice and dissect animals that become moribund during the study period, and investigate the cause of moribundity.

(iii) Usually, weigh the following organs.

a. Parent animals: Ovaries, uterus, testes, epididymis, seminal vesicle and coagulating gland, prostate, brain, liver, kidneys, adrenal glands, spleen, pituitary gland, thyroid gland, and other target organs

b.Offspring: Brain, spleen, thymus gland, and uterus

(iv) Conduct histopathological examinations of the reproductive organs and other target organs of P animals, as well as F1 animals used for breeding in the high dosage and control groups. If abnormalities are observed that are thought to be effects of the test substance, examine the moderate and low dosage groups in the same way. In addition, conduct histopathological examinations of organs and tissues of offspring in which abnormalities thought to be the effects of the test substance are noted. Refer to the results of repeated dose toxicity studies.

(v) Even after the completion of studies, preserve organs of parent animals and offspring, particularly those of the reproductive system, so that histopathological examinations can be conducted in the if necessary.

Teratogenicity studies (2-1-18)

1. Objective

The objective of these studies is to obtain scientific information regarding the effects of exposing pregnant mother animals to the test substance on the growth and development of fetuses, with special reference to teratogenicity.

2. Test animals

Use at least 2 species of animals, including 1 or more species of rodent (usually, rats) and non-rodents (usually, rabbits).

3. Administration method

(1) In general, conduct gavage continuous oral administration.

(2) Compute dosages based on weight as close as possible to the day of administration. The test substance may also be administered in feed or water if it is possible to ensure uniform dosage on the basis of blood concentration level, food consumption, etc.

4. Administration period

(1) Administer the test substance on consecutive days during a period extending at least from implantation to the last day but one prior to the expected delivery date.

(2) Establish the day on which spermatozoa are confirmed in vaginal plug or vaginal smear as gestation day 0.

5. Determining the number of animals, and establishing test groups

(1) Determining the number of animals

Use a sufficient number of pregnant animals to allow for interpretation of data.

(2) Establishing test groups

(i) Test substance administration groups

a. In addition to the control group, establish test substance dosage groups according to at least 3 dosage levels.

b. Establish dosage levels such that symptoms of test substance toxicity will be clear, and so that the NOAEL can be estimated.

c. In order to discern clearly the dose-response relationship, set the maximum dosage such that toxic effects on mother animals and fetuses, such as suppression of weight gain, can be observed, and set the minimum dosage such that no toxic effects on parent animals or fetuses are observed.

d. If no toxic effects are confirmed as a result of administration of the maximum dose that is technically possible, or 1,000 mg/kg of body weight/day of the test substance, it is not necessary to conduct studies with higher dosages than that.

(ii) Control group

a. Establish the same conditions for the control group as for the test substance administration groups, except that the test substance is not administered.

b. When a vehicle, etc. is being used for administration of the test substance, administer to the control group the same amount of vehicle as to the dosage group that is being administered the largest amount of vehicle. Establish an additional untreated control group when using a vehicle, etc. regarding which sufficient information on toxicity cannot be obtained.

6. Observation and examination

Conduct items (1)-(2) below.

(1) Parent animals

(i) General condition

a. Observe daily the general condition and gestational status of all dam.

b. Regularly weigh the animals, and determine the food consumption. (When the test substance is administered in drinking water, also measure the water consumption. Same below.) In general, weigh the animals and measure food intake on gestation day 0, on the day of dissection, and at least once every 3 days during the administration period.

c. Observation of general condition should include signs of excitation, seizure, sedation, and ambulatory irregularity, in addition to survival/fatality and outward appearance. Observations in regard to pregnancy and parturition should include abortion and premature birth.

(ii) Necropsy

a. Promptly sacrifice animals that display symptoms of abortion or premature birth, and conduct gross observations of their organs and tissues. Do the same in regard to animals that die or become moribund.

b. Sacrifice all animals on the day prior to the expected delivery date, and conduct gross examination of all organs and tissues, after removing the uterus. Weigh the extracted uteruses, and conduct examinations per (2) below.

c. Determine the number of corpora luteum. If conceptus is not observed in the uterus, conduct further examinations to determine whether implantation has occurred.

(2) Fetuses

(i) Dissect the uteruses extracted from dam. Determine the number of embryonic deaths and fetal deaths, as well as the number of surviving fetuses.

Insofar as possible, record the grounds for estimated time of death of embryos and fetuses. Determine the sex of fetuses, weigh them individually, and record these data items. Compute the mean weights of male and female fetuses in each group.

(ii) After examining all extracted fetuses for external abnormalities, examine half of the fetuses from each rat litter

for skeletal abnormalities, and examine the remainder for visceral abnormalities. Examine all rabbit fetuses for visceral abnormalities (dissect the heads of 1/2 of them, and make detailed examinations), and then examine them for skeletal abnormalities.

Mutagenicity studies (2-1-19-1~3)

1. Objective

The objective of these studies is to determine whether the test substance induces gene mutation, chromosome configuration abnormalities, or numerical abnormalities.

2. Selection of study method

Conduct reverse mutation studies using bacteria, chromosomal aberration studies using cultured mammalian cells, and micronucleus studies (using rodents) as studies of mutagenicity. If any of these studies are inapplicable, for technical or scientific reasons, it is acceptable to substitute test systems that provide similar indications. Conduct additional mutagenicity studies if further investigation is deemed necessary, on the basis of these study results.

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

1. Bacterial strains to be used

Select 1 strain from each of the following types of bacteria, and use a total of 5 strains of bacteria in the studies.

(i) *Salmonella typhimurium* TA98
(ii) *Salmonella typhimurium* TA100
(iii) *Salmonella typhimurium* TA1535
(iv) *Salmonella typhimurium* TA1537, TA97, or TA97a
(v) *Escherichia coli* WP2 uvrA, *Escherichia coli* WP2 uvrA/pKM101, or *Salmonella typhimurium* TA102

Other bacteria strains may be added, if necessitated by the properties of the test substance.

2. Dosage levels

(1) Using concentrations that will permit analysis, set at least 5 levels, at appropriate intervals.
(2) It would be preferable to conduct range findings studies for establishing the maximum concentration, using bacteria strains used in main studies. Keep growth inhibition and solubility in mind when establishing concentarations.
(3) In principle, set the concentaration at which growth is inhibited as the maximum concentration; use 5 mg/plate of substances that do not appear to inhibit growth, as the highest concentration. The concentration at which precipitation occurs may be set as the maximum dose for substances that display no growth inhibition and that are difficult to dissolve.

3. Control

Set a negative control, for which vehicle is used, and a positive control, for which a known mutagen is used, for each study.

4. Number of plates used

Use 2 or more plates for each test substance dosage level and each control.

5. Study method

(1) Conduct studies either according to the preincubation method or the plate method. A different method may be used, if there is a scientifically valid reason to do so.
(2) Whichever method is used, conduct experiments with and without metabolic activation . Use S9 mix in which

coenzymes have been added to the supernatant fraction (S9) of homogenate of livers from animals that have been appropriately treated so as to induce development of a drug metabolic enzyme system.

6. Observation

After culturing all plates for 48-72 hours at 37° C, count the number of revertant colonies per plate. At the same time, note growth inhibition and precipitation of test substance.

7. Evaluation of results

(1) If the range finding studies have been conducted regarding which sufficient information has been provided as to bacterial strains used, dosage levels, controls, and plate number, they may be used to confirm reproducibility.

(2) Make a positive judgement if there is clearly a greater number of revertant colonies as compared to the negative control group, and if reproducibility and dose dependency are confirmed. If reproducibility is not confirmed, conduct confirmation studies. If neither a positive nor a negative judgement can be clearly made, conduct confirmation studies under different experimental conditions.

8. Displaying results

Display the actual measured value for number of revertant colonies per plate and the mean for the group.

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

1. Cells used

(1) Use primary, culture and subculture cells, or established cell lines, including human cells. Examples are Chinese hamster fibroblast cell line and human peripheral blood lymphocyte.

(2) Examine the cells used in studies as to chromosome number (modal number), whether the mycoplasma is contaminated, cell cycle, etc.

2. Dosage levels

(1) Use at least 3 levels of dosage, with appropriate intervals between them (with a common ratio of 2, in general), so that they chromosome analysis can be performed.

(2) It would be desirable to conduct range findings studies in advance.

(3) The maximum dose should be the concentration at which cell proliferation is suppressed by at least 50% , regardless of test substance solubility in the culture solution. If suppression of cell proliferation by at least 50% is not observed, make 5 mg/ml or 10 mM (whichever is lower) the maximum dosage. If absolutely no suppression of proliferation is noted, and test substance precipitation is noted at the end of the treatment, the dosage at which precipitation occurs make be used as the maximum dosage.

3. Control

Set a negative control, for which vehicle is used, and a positive control, for which a known clastogen is used, for each study.

4. Number of plates used

Use 2 or more plates each for each test substance dosage level and each control.

5. Study method

(1) Use cells in proliferation phase. At first, use the short treatment method, with and without metabolic activation. Treat the cells with the test substance for 3-6 hours, and adjust chromosome specimens after approximately 1.5 cell cycles from the commencement of treatment.

(2) If negative results are obtained using the short treatment method, conduct succesive treatment for 1.5 cell cycles without metabolic activation. The ponunced delay of the cell cycle may be noticeably delayed by the test substance. If

successive treatment for longer than 1.5 cell cycles without metabolic activation is necessary, or if a longer period than 1.5 cell cycles is required for specimens preparation with metabolic activation, conduct confirmation studies as necessary. Use S9 mix in which coenzymes have been added to the supernatant fraction (S9) of homogenate of livers from animals that have been appropriately treated so as to induce drug metabolic enzymes.

6. Observations

(1) All the slide specimens, including positive and negative controls, are coded so that the treatment conditions are unknown for the examination.

(2) Observe at least 200 well-spread metaphases per dosage (100 per plate); record the number and frequency of cells with structural chromosome aberrations. Also record the number and frequency of each type of structural aberration.

(3) Record gaps separately from other aberrations, and do not include them in total aberration incidence. Define gaps as a chromosomal positions that are narrower than the chromatid width. If polyploidy or endoreduplication are observed, note the number of instances, and their incidence.

7. Evaluation of results

(1) Make a positive determination if there is clearly a greater incidence of cells with chromosomal aberration as compared to the negative control group, and if reproducibility and dose relation are confirmed.

(2) If neither a positive nor a negative determination can be clearly made, conduct confirmation studies under different experimental conditions.

8. Displaying results

Display all observation data on each plate, together with mean group values. Display data concerning cell proliferation in the dosage groups and negative control group. Clearly record dosages at which precipitation of test substance is observed.

Micronucleus studies (2-1-19-3)

1. Types of animals

In general, use either mice or rats when using red blood cells from bone marrow; use mice when using red blood cells from red blood cells from peripheral blood. However, other appropriate types of animals may be used. The studies may be conducted with males only if no clear sex difference is observed as to toxicity.

2. Number of animals

Use 5 or more animals per group.

3. Administration route

Use gavage or intraperitoneal administration.

4. Number of administrations

Conduct either single or multiple administration.

5. Dosage levels

(1) Establish at least 3 levels of dosage, at appropriate intervals.

(2) Set the maximum dosage as the dosage at which cytotoxicity is confirmed by a decline in immature red blood cells, etc. in bone marrow, or at which symptoms of toxicity are observed, or beyond which it is expected that any dosage would be lethal.

(3) Set 2,000 mg/kg as the maximum dosage if toxicity is not confirmed.

6. Control

Set a negative control, for which vehicle is used, and a positive control, for which a known micronucleus inducer is used, for each study.

7. Methods

(1) Use red blood cells from bone marrow or peripheral blood.

(2) Adjust samples twice at appropriate times following administration of the test substance, in the case of single administration, and once at an appropriate time following administration of the test substance, in the case of multiple administration.

(3) If it is possible to confirm the time at which susceptibility is maximized, it is satisfactory to adjust samples only once, even following single administration.

8. Observations

(1) All the slide specimens, including positive and negative controls, are coded so that the treatment conditions are unknown for the examination.

(2) Observe at least 2,000 immature red blood cells per individual, and record the incidence of cells that have micronuclei.

(3) Observe at least 200 red blood cells per individual, in the case of bone marrow, and at least 1,000 per individual, in the case of peripheral blood, and record the ration of immature red blood cells to total red blood cells.

9. Evaluation of results

(1) Make a positive determination if dose relation is noted in the increase in the number of cells with micronuclei, or if there is a clear increase in the number of cells with micronuclei in any administration groups.

(2) If neither a positive nor a negative determination can be clearly made, conduct confirmation studies under different experimental conditions.

10. Displaying results

Display observational data for each individual, and the average values for each group.

Pharmacology studies (2-2-1)

1. Objective

The objective of these studies is to pharmacologically analyze the acutely toxic effects of the test substance, to clarify the possibility of the occurrence of symptoms of acute intoxication, as well as their characteristics, and to obtain useful information on mechanisms of and treatment for acute intoxication. Study items and methods should be selected that are appropriate for the test substance's use pattarn, the characteristic ways in which its toxicity manifests, and its physical characteristics.

2. Test animals

Use young male and female animals, such as mice, rats, guinea pigs, rabbits, and dogs, as appropriate for the relevant study items.

3. Study plan basics

(1) Administration route

In general choose an administration route by which acute exposure can be anticipated.

(2) Number of administrations

A single dose is given in general in test using whole animals.

(3) Establishing dosages

Establish administration groups such that the no observed adverse effect level (NOAEL) and dose relation of toxic

response will be clear in each study. Set the maximum dose as the dosage at which fatalities occur. With oral administration, it is not necessary to perform studies with dosages higher than 2,000 mg/kg of body weight.

(4) Control group

Establish a negative (vehicle) control group for each study.

(5) Observation (examination) period

Observations are performed at times which will demonstrate the onset of toxic response, maximum effects and their disappearance.

(6) When using anesthetized animals

Take changes in absorption of the test substance due to anesthetic into account when conducting studies.

(7) When using extracted organ (tissue) samples

Establish a negative (vehicle) control group, and also establish a group receiving a concentration such that NOAEL and dose relation will be clear. It would be desirable to set concentration according to estimated tissue concentrations in the active site. Employ appropriate organic vehicles and surfactants for test substances of low solubility, and apply them to liquid nutrients in a dissolved or emulsified state. Also examine the reversibility of effects.

(8) Number of examples

The number of animals per group should be such that appropriate statistics can be obtained. However, use at least 3 animals per group for items regarding which time-lapse tracking is important, to confirm reproducibility.

4. Examination items

Conduct examinations in regard to the following items, in order to ascertain toxic response on the basis of acute pharmacological effects. Not all the items are required for fulfilling the principles of these studies. When selecting examination items, refer to the items listed below, and base decisions on information obtained from results of other toxicity studies.

Select examination methods that facilitate scientifically correct evaluation of toxic effects, based on the pharmacological effects of the test substance.

(1) Items that should usually be conducted

(i) Observation of symptoms

Observe systems such that they are ascertained objectively, quantitatively, and temporally.

(ii) Central nervous system

Effects on spontaneous movement, convulsions, etc.

(iii) Respiratory and circulatory systems

Effects on respiration, blood pressure, heart rate, and electrocardiagram

(iv) Renal functions

Urine volume, electrolyte concentration in urine, urine osmotic pressure (specific gravity), etc.

(2) Items to be added and conducted as deemed necessary on the basis of results obtained in (1) above, and information obtained from other toxicity study results

(i) Autonomic nervous system

Effects on pupil diameter, nictitating membrane, extracted ductus deferens, etc.

(ii) Skeletal muscle

Effects on grip strength, extracted skeletal muscle, etc.

(iii) Blood system

Hemolytic effects, effects on clotting functions, etc.

(iv) Digestive system

Effects on peristalsis, extracted intestines, agonist contraction, etc.

(v) Other

Other items deemed necessary on the basis of observation of symptoms and other toxicity studies.

Studies of metabolic fate in animals (2-3-1)

1. Objective

The objective of these studies is to contribute to evaluation, etc. of study results regarding toxicity of agricultural chemicals, by administering test substances to animals and obtaining scientific findings concerning their pharmacokinetics (absorption, distribution, excretion, metabolism, etc.

2. Test substance

Use compounds, of a high degree of purity, that are the active ingredients, etc. of agricultural chemicals, and that are either labeled or unlabeled with radioactive istopes. Verify their source, purity and stability. Clarify the method of synthesis, labeled nuclide, labeled position, and specific radioactivity of the isotope labels.

3. Test animals

(1) Use young adult animals of a single species (usually, rats).
(2) It would be preferable to use animals of the same strain as those used in the 1-year repeated dose oral toxicity and carcinogenicity studies.
(3) In principle, use both male and female animals.
(4) If there are conspicuous differences between rodents and non-rodents as regards the target organs or degree of toxic symptoms, it would be desirable to use non-rodents in addition. Pregnant animals may also be used, if necessary.

4. Administration method

(1) Administration route

Use oral administration, in general. If necessary, supplement with studies using intravenous administration, etc.

(2) Number of administrations and administration period

Conduct single administration, in general. Consider conducting repeated dose if accumulation is anticipated. In case of repeated dose, estimate the test substance's *in vivo* steady state and accumulation, and allow for sufficient intervals (usually once per day) and administration period (in general, 14 days).

5. Determining the number of animals, and establishing test groups

(1) Determining the number of animals

Use an appropriate number of animals (in general, at least 4 in each dosage group) for achieving test objectives, taking into account individual differences, and the number of specimens required for observations and measurements.

(2) Establishing test groups

(i) Use at least 2 different dosages for single administration. When establishing the 2 dosage levels, use guidelines whereby the high dose is the dosage at which some toxic effects are observed, while the low dose is the dosage at which no toxic effects are observed.

(ii) For repeated dose, use only the low dose in general, and use the high dose if necessary.

6. Items to consider

Items to consider in regard to adsorption, distribution, excretion, and metabolism are usually as follows.

(1) Absorption

Obtain information regarding the amount of the test substance absorbed, and the absorption speed. These are obtained based on analyses of estimates, etc. of amounts excreted and blood concentrations (serum concentration, plasma concentration, and total blood concentration).

(2) Distribution

Obtain information concerning the distribution (concentration and distribution rate), temporal change, and accumulation of the test substance and metabolites in major organs and tissues (including organs and tissues in which toxic effects have been noted) at multiple points in time, as appropriate, including Tmax and at the end of the

examination of excretion.

(3) Excretion

Measure the amount of the test substance and metabolites excreted in excrement and exhalation, and obtain from these the total amount excreted. Also obtain information concerning the excretion route, as well as the degree and speed of excretion. Measurements of amount excreted should be measured over time, until 7 days after administration, or until 90% of the amount administered has been excreted, which ever occurs sooner. If necessary, measure the amount excreted in bile and milk, as well.

(4) Metabolism

Use appropriate methods to identify and quantify the test substance. Also obtain information concerning the excretion route, as well as the degree and speed of excretion.

(5) Other

It would be desirable to investigate, insofar as possible, in order to elucidate, for example, various items suggesting relationships between biopolymer bonding and toxicity, organs and tissues that contribute to metabolism, etc.

Studies of metabolic fate in plants (2-4-1)

1. Objective

The objective of these studies is to obtain scientific information regarding the absorption and translocation of the test substance inside plant bodies, as well as concerning the major metabolic routes and the amounts of metabolites. Together with results of studies of fate in plants, the studies should also contribute to confirmation of dissimilarities between metabolites found in animals and plants, and to determinations regarding substances analyzed in studies of residue in crops.

2. Test substance

Use compounds, of a high degree of purity, that are the active ingredients, etc. of agricultural chemicals, and that are either labeled or unlabeled with radioactive istopes. Verify their source, purity and stability.

Clarify the method of synthesis, labeled nuclide, labeled position, and specific radioactivity of the isotope labels.

3. Test plants

It would be desirable to conduct studies in which plants are cultivated under conditions close to those under which the plants are usually cultivated.

4. Treatment method

(1) Use a test substance that is representative of the formulation for which registration is intended, or has been adjusted so as to be similar in form or composition.

(2) In general, carry out treatment according to methods of use, usage period, and amount used that are relevant to the registration application.

(3) If multiple differing application methods are anticipated, it would be desirable, in general, to conduct multiple studies.

5. Collecting samples

(1) In general, gather samples when the relevant crops are harvested. However, if the period from application to harvest is lengthy, and it would be difficult to elucidate metabolic route merely upon observation at harvest time, data may be collected multiple times from the first to last period of application.

(2) It is desirable to gather samples at various times during the harvest period, in the case of crops that have long harvest periods.

(3) In general, distinguish between samples gathered from various parts of plants, such as roots, leaves, stems, or fruit, and between treated and untreated parts, if relevant.

6. Analysis

(1) Determine in each of the gathered and separated samples the amount of metabolites (includes unchanged test substance; same below) present, etc.

(2) As regards the treated parts of plants, analyze metabolites that are left on the surface separately, insofar as possible, from metabolites that permeate the plant body.

(3) In general, analyze samples promptly. Adopt appropriate methods for minimizing decomposition of metabolites when storing plant samples and liquid extracts. It is also necessary to ensure that changes in metabolites can be ascertained during the storage period.

7. Metabolite identification, etc.

It is desirable, in general, to identify metabolites as follows:

(1) Identify or determine the chemical properties of metabolites that account for 10% or more of the total residual in the sample parts, equivalent to , if converted into test substance, represent a concentration of 0.01 mg/kg of all metabolites and similar substances.

(2) Insofar as technically possible, identify metabolites that account for 10% or more of the total residual in the sample parts, equivalent to test substance, represent a concentration of 0.05 mg/kg of all metabolites and similar substances.

(3) Insofar as technically possible, endeavor to identify metabolites characteristic of plant bodies (in the case of conjugates, their aglycon portions), even when the quantity available is insufficient for this purpose.

(4) It would be desirable to identify metabolites with residuals of 50% or more, and to determine the chemical properties of those that represent 70% or more of the total residual, when the radioactive residual concentration is 0.01 mg/kg equivalent to test substance.

(5) Determine the chemical properties of metabolites that represent 10% or more of the total residual, and of extracted residual components that are generated in concentrations of 0.05 mg/kg or more, by treating them with surfactants, enzymes, acids, bases, etc.

(6) It would be desirable to determine the characteristics of metabolites by converting them to common molecular components, insofar as possible, when the radioactive residual concentration is 0.01 mg/kg equivalent to test substance, and that represent less than 10% of the total residual.

Studies of fate in soil (2-5-1~3)

Studies of fate in flooded aerobic soil (2-5-1)

1. Objective

The objective of these studies is to obtain scientific information regarding the main metabolic pathway of the test substance in flooded soil under aerobic conditions, the types of metabolites, and the mass balance of the test substance. These studies contribute to the analysis of the results of animal and plant metabolism studies, and to the selection of substances to be analyzed in soil residue studies.

2. Test substance

Use compounds, of a high purity, that are the active ingredients, etc. of agricultural chemicals, and that are either labeled with radioactive isotopes or unlabeled. Verify their source, purity and stability.

Clarify the method of synthesis, labeled nuclide, labeled position, and specific radioactivity of the isotope labels.

3. Test soil

(1) Use fresh surface soil collected from rice paddies.

(2) Use soil that is fresh soil, or soil that has been subjected to minimal air-drying at room temperature, if necessary; pass it through a 2 mm sieve (in general) prior to use. Do not air dry the soil completely.

(3) It would be desirable to use sterilized soil additionally, in order to observe the effects of microorganisms.

4. Test conditions

(1) The soil depth should be at least 5 cm. Flood the soil in water depth of at least 1 cm.

(2) The studies should be conducted under dark conditions, and in general at 25° C (± 2°).

(3) The studies should be conducted, in general, under conditions such that gases are exchanged freely, and that test substance etc. released in the gas phase during the study period can be recovered.

(4) After preliminary incubation of soil (preincubation) for at least 2 weeks under the same conditions as those maintained during the test period and after confirming reduction zone formation, treat with test subsutance.

5. Test procedures

(1) Treatment

(i) Dissolve the test substance in water or a small amount of organic solvent (e.g.,acetone, etc.); after the test system has been treated once, stir and shake, etc. so that there is a uniform distribution of test substance in the flooded water.

(ii) Set a level of the treatment concentration, using as a standard the concentration when the maximum conventional application of the test substance (or active ingredient) is applied once and distributed uniformly, in soil with a depth of 10 cm. However, if the conventional application is so low as to interfere with analyses, etc., use a concentration within the range enabling analysis.

(2) Test period

Set a period sufficient for ascertaining disappearance of the test substance, as well as formation and disappearance of its main metabolites. Set 6 months as the maximum period.

(3) Collecting samples for analysis

Collect samples of test system soil, water, and gas at least 6 times, including immediately after treatment and at the end of the study period, so as to enable appropriate analysis of the formation and disappearance of metabolites, etc. (including unchanged test substance; same below). By means of decantation method, separate water from soil.

(4) Analysis

In general, analyze samples promptly. Adopt appropriate methods for minimizing decomposition of metabolites when storing soil samples, extract solution, etc. It is also necessary to ensure that changes of metabolites can be ascertained during the storage period.

6. Points to investigate

Points to investigate in regard to all steps of distribution and metabolism are as follows.

(1) Distribution

Obtain information regarding time-dependent changes in the distribution of the test substance, etc. in soil, water, and gas phases of test system, and clarify the mass balance of the substance. If bound residues have formed after soil extraction, obtain information on the ratio at which they form.

(2) Metabolism

Identify and/or characterize test substance and main metabolites present in test system soil, water, and gas phase; characterize and quantify them. Obtain information regarding metabolic pathway. Also obtain information regarding disappearance of test substance, as well as, when possible, disappearance of the main metabolites. Characterize the properties of bound residues that form after soil extraction.

Studies of fate in aerobic soil (2-5-2)

1. Objective

The objective of these studies is to obtain information regarding the main metabolic pathways of the test substance in soil under aerobic conditions, the types of metabolites, and the mass balance of the test substance. These studies contribute to the analysis of the results of animal and plant metabolism studies, and to the selection of substances to be analyzed in

soil residue studies.

2. Test substance

Use compounds, of a high purity, that are the active ingredients, etc. of agricultural chemicals, and that are either labeled with radioactive isotopes or unlabeled . Verify their source, purity and stability. Clarify the method of synthesis, labeled nuclide, labeled position, and specific radioactivity of labeled substance.

3. Test soil

(1) Use fresh surface soil collected from fields.
(2) Use soil that is fresh soil, or soil that has been subjected to minimal air-drying at room temperature, if necessary; in general pass it through a 2 mm sieve prior to use. Do not air dry the soil completely.
(3) It would be desirable to use sterilized soil additionally, in order to observe the effects of microorganisms.

4. Test conditions

(1) The soil depth should be 1-5 cm.
(2) Maintain the water content of the soil at 40-60% of its maximum water holding capacity.
(3) The studies should be conducted under dark conditions, and in general at 25° C (± 2°).
(4) The studies should be conducted, in general, under conditions such that gases are exchanged freely, and such that test substance etc. that is released in the gas phase during the study period can be recovered.
(5) After preliminary incubation of soil for at least 2 weeks under the same conditions as those maintained during the study period, treat with test substance.

5. Test procedure

(1) Treatment
(i) Dissolve the test substance in water or a small amount of organic solvent (e.g., acetone, etc.); after the test system has been treated once, stir and shake, etc. so that there is uniform distribution of test substance in the soil.
(ii) Set a level of the treatment concentration , using as a standard the concentration when the maximum conventional application of the test substance (or active ingredient) is applied once and distributed uniformly, in soil with a depth of 10 cm. However, if the conventional application is so low as to interfere with analyses, etc., use a concentration within the range enabling analysis.

(2) Test period

Set a period sufficient for ascertaining disappearance of the test substance, as well as formation and disappearance of its main metabolites. Set 6 months as the maximum period.

(3) Collecting samples for analysis

Collect samples of test system soil and gas at least 6 times, including immediately after treatment and at the end of the study period, so as to enable appropriate analysis of the formation and disappearance of metabolites, etc. (includes unchanged test substance; same below).

(4) Analysis

In general, analyze samples promptly. Adopt appropriate methods for minimizing decomposition of metabolites when storing soil samples, extract solution, etc. It is also necessary to ensure that changes of metabolites can be ascertained during the storage period.

6. Points to investigate

Points to investigate in regard to all steps of distribution and metabolism are as follows.

(1) Distribution

Obtain information regarding time-dependent changes in the distribution of the test substance and its metabolites, etc. in soil and gas phase of test system, and clarify the mass balance of the substance. If bound residues have formed after soil extraction, obtain information on the ratio at which they form.

(2) Metabolism

Identify and/or characterize test substance and main metabolites present in test system soil and gas phase; characterize and quantify them. Obtain information regarding metabolic pathways. Also obtain information regarding disappearance of test substance, as well as, when possible, disappearance of the main metabolites. Characterize the properties of bound residues after soil extraction.

Studies of fate in anaerobic soil (2-5-3)

1. Objective

The objective of these studies is to obtain scientific information regarding the main metabolic pathways of the test substance in soil under anaerobic conditions, the types of metabolites, and the mass balance of the test substance. These studies contribute to the analysis of the results of animal and plant metabolism studies, and to the selection of substances to be analyzed in soil residue studies.

2. Test substance

Use compounds, of a high degree of purity, that are the active ingredients, etc. of agricultural chemicals, and that are either labeled with radioactive isotopes or unlabeled . Verify their source, purity and stability prior to obtaining them. Clarify the method of synthesis, labeled nuclide, labeled position, and specific radioactivity of the labeled substance.

3. Test soil

(1) Use fresh surface soil collected from fields.
(2) Use soil that is fresh, or soil that has been subjected to minimal air-drying at room temperature, if necessary; in general pass it through a 2 mm sieve prior to use. Do not air dry the soil completely.

4. Test conditions

(1) The soil depth should be at least 5 cm.
(2) Flood the soil so that the water is at least 1 cm deep.
(3) The studies should be conducted under dark conditions, and in general at 25° C (± 2°).
(4) The studies should be conducted, in general, under anaerobic conditions such that anaerobic condition is maintained by free exchange of inert gases, and such that test substance etc. that is released in the gas phase during the study period can be recovered.
(5) After preliminary incubation(preincubation) for at least 2 weeks under the same conditions as those maintained during the test period and after confirming reduction zone formulation, treat with test substance.

5. Test prpcedures

(1) Treatment
(i) Dissolve the test substance in water or a small amount of organic vehicle (acetone, etc.); after the test system has been treated once, stir and shake, etc. so that there is uniform distribution of test substance in the flooded water.
(ii) Set a level of the treatment concentration, using as a standard the concentration when the maximum conventional application of the test substance (or active ingredient) is applied once and distributed uniformly, in soil with a depth of 10 cm. However, if the conventional application is so low as to interfere with analyses, etc., use a concentration within the range enabling analysis.

(2) Test period

Set a period sufficient for ascertaining disappearance of the test substance, as well as formation and disappearance of its main metabolites. Set 6 months as the maximum period.

(3) Collecting samples for analysis

Collect samples of test system soil, water, and gas at least 6 times, including immediately after treatment and at the end of the study period, so as to enable appropriate analysis of the formation and disappearance of metabolites, etc. (includes unchanged test substance; same below). By means of decantation method, separate water from soil.

(4) Analysis

In general, analyze samples promptly. Adopt appropriate methods for minimizing decomposition of metabolites when storing soil samples, extract solution, etc. It is also necessary to ensure that changes of metabolites can be ascertained during the storage period.

6. Points to investigate

Investigate to consider in regard to all steps of distribution and metabolism are as follows.

(1) Distribution

Obtain information regarding time-dependent changes in the distribution of the test substance, etc. present in test system soil, water, and gas phases, and clarify the mass balance of the substance. If bound residues have formed after soil extraction, obtain information on the ratio at which they form.

(2) Metabolism

Identify and/or characterize test substance and main metabolites present in test system soil, water, and gas phases; quantify them. Obtain information regarding metabolic pathways. Also obtain information regarding disappearance of test substance, as well as, when possible, disappearance of the main metabolites. Characterize the properties of bound residues that form after soil extraction.

Studies of fate in water (2-6-1, 2)

Studies of hydrolytic fate (2-6-1)

1. Objective

The objective of these studies is to obtain information regarding the hydrolytic fate of test substance in water, their main metabolic routes, the types of decomposition substances formed, and the mass balance of the test substance. These studies contribute to the analysis of the results of animal and plant metabolism studies and to the selection of substances to be analyzed in water residue studies.

2. Test substance

Use compounds, of a high degree of purity, that are the active ingredients, etc. of agricultural chemicals, and that are either labeled with radioactive isotopes or unlabeled . Verify their source, purity and stability. Clarify the method of synthesis, labeled nuclide, labeled position, and specific radioactivity of the labeled substance.

3. Test water

Use buffer solutions of pH 4.0, 7.0, and 9.0.

4. Test conditions

(1) Conduct studies at 25 ± 1° C.

(2) Eliminate causes of decomposition other than hydrolysis (light, oxygen, etc.).

(3) Sterilize test water and test containers.

5. Test procedures

(1) Treatment

Use a single test concentration of the test substance. In general, select 0.01 M, , 1/2 or lower of the water solubility, whichever is lower.

(2) Test period

Set a period sufficient for ascertaining disappearance of the test substance, as well as formation and disappearance of its main decomposition substances. Set 30 days as the maximum period. At the end of the study period, it would be desirable to check whether sterile conditions have been maintained in the test system.

(3) Collecting samples for analysis

Collect samples (water and gas) at least 7 times, including immediately after treatment and at the end of the study period, so as to enable appropriate analysis of the disappearance of test substance and the formation and disappearance of metabolites, etc.

(4) Analysis

(i) Analyze samples promptly after collecting them.

(ii.) Adopt appropriate methods for minimizing degradation of decomposition products when storing samples, extract solution, etc. It is also necessary to ensure that changes of decomposition products can be ascertained during the storage period.

6. Points to investigate

Points to investigate are usually as follows.

(1) Mass Balance

Clarify the mass balance.

(2) Decomposition products, etc.

Use appropriate methods to identify and/or characterize test substance and main decomposition products present in water and quantify them. If volatile substances are formed, identify and/or characterize and quantify them. Obtain information regarding hydrolytic pathways, as well as fate of the main decomposition products.

(3) Decomposition rate

Obtain information regarding disappearance of the test substance, as well as, when possible, rate of the main decomposition products.

Studies of photolytic fate in water (2-6-2)

1. Objective

The objective of these studies is to obtain information regarding the photolytic fate in water of the test substance under light, as well as their main metabolic pathways, the types of substances formed through decomposition, and the mass balance of the test substance. These studies contribute to the analysis of the results of animal and plant metabolism studies and to the selection of substances to be analyzed in water residue studies, etc.

2. Test substance

Use compounds, of a high degree of purity, that are the active ingredients, etc. of agricultural chemicals, and that are either labeled with radioactive isotopes or unlabeled. Verify their source, purity and stability. Clarify the method of synthesis, labeled nuclide, labeled position, and specific radioactivity of the isotope labels.

3. Test water

Use natural water and distilled water (or buffer solution).

4. Test conditions

(1) Use, as a test light source, artificial lighting that is similar to sunlight that would reach the ground, in terms of wavelength distribution. Conduct continuous irradiation. Measure the wavelength distribution and light intensity of on the sample stage.

(2) The surfaces of the test containers that receive incident light must be made of materials that will not absorb the light described above.

(3) Sterilize test water and test containers. When using natural water, sterilize in such a way as not to change its components.

(4) Conduct studies at 25 ± 2° C.

5. Test procedures

(1) Treatment

Use a single test concentration of the test substance. Use a concentration that is less than 1/2 of the concentaration of the water solubility, and which is sufficient for analyses of the test substance disappearance rate and fate of decomposition products.

(2) Test period

Set the study period such that disappearance of the test substance and the fate of the main decomposition products can be ascertained. The study period need not to exceed 30- day equivalent to sunlight at 35° north latitude (Tokyo); spring (from April to June). Following completion of the study, it is desirable to confirm that sterile conditions have been maintained in the test system.

(3) Collecting samples for analysis

Collect samples (water and gas) at least 7 times, including immediately after treatment and at the end of the study period, so as to enable appropriate analysis of the disappearance of the test substance, as well as the formation and disappearance and fate of the main decomposition products.

(4) When conducting studies, establishing a sample under dark area as a control.

(5) Analysis

(i) Analyze samples promptly after collecting them.

(ii) Adopt appropriate methods for minimizing degradation of decomposition products when storing samples, extract solution, etc. It is also necessary to ensure that changes of decomposition products can be ascertained during the storage period.

6. Points to investigate

Points to investigate are usually as follows.

(1) Mass Balance

Clarify the mass balance.

(2) Decomposition products, etc.

Use appropriate methods to identify and/or characterize test substance and main decomposition products present in water; quantify them. If volatile substances are formed, identify and/or characterize and quantify them. Obtain information regarding decomposition pathways, as well as fate of the main decomposition products.

(3) Decomposition rate

Obtain information regarding disappearance of test substance, as well as, when possible, fate of the decomposition products.

Aquatic organism effect studies (2-7-1~3)

Fish acute toxicity studies (2-7-1)

1. Objective

The objective of these studies is to establish safe use methods of using agricultural chemicals by obtaining scientific information regarding the short-term effects of the test substance on fish.

2. Definitions

(1) Death: A fish is considered dead if there is no observable movement (of the gill covers, etc.), and there is there is no response when the tail is touched.

(2) Median lethal concentration (LC50): The test substance concentration at which 50% of the test animals die during the exposure period.

(3) No observed effect concentration (NOEC): The maximum concentration at which no effects are observed as compared to the control.

(4) Test substance: TGAI or formulation of the agricultural chemical to be studied.

(5) Standard substance: Substance used for confirming the reproducibility of test conditions.

(6) Test chemical: Test substance and standard substance used in the studies.

(7) Static test: A test that is conducted according to a system in which the test solution is not exchanged during the exposure period.

(8) Semi-static test: A test that is conducted according to a system in which the test solution is exchanged in each container in each fixed period.

(9) Flow-through test: A test that is conducted according to a system in which test solution is supplied continuously.

3. Test organisms

(1) Organism species

(i) Select the test fish from the table at the end of this section.

(ii) The LC50 of the standard substance should be recommended to confirm.

(2) Acclimatization

(i) The test fish must be acquired by the 12th day prior to their use in studies, and maintained from that time.

(ii) Dip the fish in a medicated bath when received, if necessary.

(iii) The fish must be acclimated to the same environmental conditions (water quality, etc.) under which studies will be conducted for at least 9 days prior to use in studies.

(iv) The fish should be fed at least 5 times per week, but must not be fed for 24 hours prior to use for the study.

(v) Acclimatization should be conducted under the following conditions, and record the mortality rate.

a. If during the 7-day period following the stabilization period (the 2 days following the commencement of acclimatization) the mortality rate exceeds 10% of the individuals in a group, that group should be excluded.

b. If the group mortality rate is 5-10%, and is still 5% or more after acclimatization for 7 days, exclude the group, or continue acclimatization until the mortality rate falls below 5%.

c. If the group mortality rate is less than 5%, the fish in that group may be used in the studies.

4. Exposure method.

Conduct the studies under static, semi-static, and flow-through conditions.

5. Exposure period

Conduct the studies for 96 hours.

6. The number of test fish, and designing experimental plots

(1) Number of test fish

Use at least 7 fish in each experimental group.

(2) Design of experimental groups

(i) Establishing test concentration groups

a. Set groups of at least 5 different concentrations in a geometrical series.

b. Determine test concentrations and a factor for concentration on the basis of preliminary study results.

c. It would be desirable to include within the concentration range the concentrations at which all test fishes die, and that at which there are no fatalities, as 1 level each, and at least 2 more levels at which some of the fishes die.

(ii) Design of control groups

a. Establish as a control a group that is not treated with the test substance.

b. When using a solubilizing agent to adjust the test liquid concentrate, establish a solubilizing agent control group, treated with the maximum concentration of solubilizing agent to be used in the study.

7. Preparation of the test solution

The test solution is prepared as described below. It would be desirable to prepare the test solution and test concentrate immediately prior to their use in the study.

(1) When using TGAI as the test substance

(i) When using a water soluble TGAI, dissolve it in the water that is to be used to dilute the test substance, and

prepare the test solution or test solution concentrate.

(ii) When using a TGAI that is not water soluble, use mechanical means to disperse the test substance, and prepare the test solution or test solution concentrate, or prepare the test solution concentrate with a solubilizing agent, such as organic vehicle, emulsifier, or dispersant. Use a solubilizing agent that is of low toxicity to fish, which has not been noted to have adverse effects on fish at the concentration used in the study, and which does not change the properties of test substance.

(iii) The solubilizing agent concentration in the test solution should not exceed 100 mg/l (or 0.1 ml/l).

(2) When using a formulation as the test substance

Add the formulation to the dilution water, and stir. Prepare the test solution or test solution concentrate. Do not use a solubilizing agent to prepare the formulation.

8. Environmental conditions

(1) Population of test fish

(i) In static and semi-static studies, it is necessary to use at least 1 L of test solution per gram of test fish body weight.

(ii) A still higher population may be used in flow-through studies.

(2) Water temperature

Set the temperature according to the species of test fish, as shown in the table below, within a variance range of ± 2° C.

(3) Illumination

Illuminate for 12-16 hours.

(4) Feeding

Do not feed during the exposure period.

(5) Dilution water

(i) Do not use water that contains hazardous substances for the study. Use water after its quality has been demonstrated to be favorable to the survival and development of fish, from the same source as the water in which they were bred.

(ii) Use dechlorinated tap water, natural water supplies, or reconstituted water.

(iii) Aerate the water to air sufficiently prior to use, and prepare the temperature.

(6) Dissolved oxygen concentration

Maintain the dissolved oxygen concentration as at least 60% of the saturate concentration. Gentle aeration applied , as necessary.

(7) pH

Do not adjust the pH of the test solution.

9. Observation and measurement

(1) Observation of the general condition of test fish

At the very least, observe the general condition of test fish at the 24, 48, 72, and 96 hours after the commencement of exposure, and keep records. Promptly remove dead fish from the test system. Also record any abnormalities observed.

(2) Measuring test substance concentration.

(i) When using TGAI as the test substance, measure the concentration of test substance in each test concentration group at least at the commencement and end of exposure, and prior to and following water changes.

(ii) During the exposure period, the test substance concentration should be 80% or more higher than the nominal concentration.

(3) Measurement of environmental conditions

(i) Confirm the quality of the dilution water prior to the study.

(ii) Measure water temperature, dissolved oxygen concentration, and pH of the test solution in each test group at least at the commencement and end of exposure, and prior to and following water changes.

10. Method of processing results

(1) Use an established method for computing LC_{50}, based on the mortality rate results for each concentration.

(2) If the measured values for test substance concentration fluctuate ± 20% or more above the nominal concentration, compute LC_{50} on the basis of the mean measured concentration.

11. Reporting

(1) In regard to the test substance

(2) In regard to the test fish

Species name, water source, breeding method, acclimatization, number of test fish, length and weight of fish, LC_{50} with standard substance, etc.

(3) In regard to study method

Exposure conditions, environmental conditions, items for observation and measurement, etc.

(4) In regard to study results

(i) LC50, and its 95% confidence limit (at the time of each observation, if possible)

(ii) Method of calculating LC_{50}

(iii) NOEC (If NOEC values have not been obtained, record the reason.)

(iv) The cumulative mortality rate in each test group at the time of each observation

(v) A graph of the concentration/mortality curve at the end of the exposure period

(vi) Abnormal symptoms and responses of test fish

(vii) Measured values of test substance concentration (only when using TGAI as the test substance)

(viii) Results of measurement of environmental conditions

Water quality, dissolved oxygen concentration, pH, etc.

(ix) Other items

Test solution conditions, and other items that might affect study results

12. Study validity

(1) The mortality rate must not exceed 10% in control groups at the end of the exposure period. If less than 10 fish are used, it is unacceptable for more than 1 of them to die.

(2) The dissolved oxygen concentration must be maintained as at least 60% of the saturate concentrate.

Table: Conditions and temperatures to be set according to test organism species

Fish Species	Set Temperature (° C)	Test Fish Length (cm)
Cyprinus carpio	20-24	5.0 ± 1.0
Oryzias latipes	21-25	2.0 ± 1.0
Lepomis macrochirus	21-25	3.0 ± 1.0
Oncorhynchus mykiss	13-17	5.0 ± 1.0
Poecilia reticulata	21-25	2.0 ± 1.0
Brachydanio rerio	21-25	2.0 ± 1.0
Pimephales promelas	21-25	2.0 ± 1.0

Daphnia acute immobilization studies (2-7-2-1)

1. Objective

The objective of these studies is to establish safe use methods of using agricultural chemicals by obtaining scientific information regarding the short-term effects of the test substances on crustaceans.

2. Definitions

(1) Immobilization: If there is no swimming movement at all in a test solution for 15 seconds after the test container is

shaken lightly, immobilization is considered to have occurred.

(2) Median effect concentration (EC_{50}): The test substance concentration at which 50% of the test organisms are immobilized during the exposure period.

(3) No observed effect concentration (NOEC): The maximum concentration at which no effects are observed as compared with the control.

(4) Test substance: TGAI or formulation of the agricultural chemical to be tested.

(5) Standard substance: Substance used for confirming the reproducibility of study conditions.

(6) Test chemical: Test substance and standard substance used in the studies.

(7) Static test: A test that is conducted according to a system in which the test solution is not changed during the exposure period.

(8) Semi-static test: A test that is conducted according to a system in which the test solution is changed in each container during each fixed period.

(9) Flow-through test: A test that is conducted according to a system in which test solution is supplied continuously.

3. Test organisms

(1) Organism species

(i) Use *Daphnia magna*. However, other species of daphnia may be used if equivalent study results can be obtained thereby.

(ii) Use test organisms of known history (the supplier, breeding methods, etc.).

(iii) It would be desirable to confirm the EC_{50} of a standard substance.

(2) Life stage

Use individuals that are no more than 24 hours old (hereinafter referred to as "young daphnia").

(3) Breeding of parent *daphnia*

The parent daphnia used to obtain young daphnia should be bred for a fixed period under conditions as close to the test environmental conditions as possible (same water quality as that of the dilution water used in the studies, water temperature, etc.). Daphnia should be healthy and in their reproductive prime (usually 2-4 weeks old).

4. Exposure method.

Conduct the studies under static, semi-static, and flow-through conditions.

5. Exposure period

Conduct the studies for 48 hours. However, this may be shortened to 24 hours, depending on the species of test organism.

6. Determining the number of test organisms, and establishing experimental groups

(1) Number of test organisms

Use at least 20 test organisms in each test group. Divide the organisms into numbers of individuals as necessary for observation.

(2) Experimental groups

(i) Test concentration setting

a. Establish at least 5 different concentrations in a geometrical series.

b. Determine test concentrations and a factor for concentration on the basis of preliminary test results.

c. It would be desirable to include within the concentration range the concentrations at which all test organisms are immobilized, and that at which there is no immobilization, as 1 level each, and at least 2 more levels at which some of the organisms are immobilized.

(ii) Control groups

a. Design as a control a group that is not treated with the test substance.

b. When using a solubilizing agent to adjust the test solution concentrate, design a solubilizing agent control group, treated with the maximum concentration of solubilizing agent to be used in the study.

7. Preparation the test solution

The test solution is prepared as described below. It would be desirable to prepare the test solution and test solution concentrate immediately prior to their use in the study.

(1) When using TGAI as the test substance

(i) When using a readily water soluble TGAI, dissolve it in the water that is to be used to dilute the test substance, and prepare the test solution or test solution concentrate.

(ii) When using a TGAI that is not water soluble, use mechanical means to disperse the test substance, and prepare the test solution or test solution concentrate, or prepare the test solution concentrate with a solubilizing agent, such as organic solvent, emulsifier, or dispersing agent. Use a solubilizing agent that is of low toxicity to the organisms, which has not been noted to have adverse effects on the organisms at the concentration used in the study, and which does not change the properties of the test substance.

(iii) The solubilizing agent concentration in the test solution should not exceed 100 mg/l (or 0.1 ml/l).

(2) When using a formulation as the test substance

Add the formulation to the dilution water, and stir. Prepare the test solution or test solution concentrate. Do not use a solubilizing agent to prepare the formulation.

8. Environmental conditions

(1) Test solution volume

At least 5 ml per individual daphnia.

(2) Water temperature

Set the temperature at 20° C, with the range of ± 1° C during the study period.

(3) Light

Light for 12-16 hours.

(4) Feeding

Do not feed during the exposure period.

(5) Dilution water

(i) Do not use water that contains hazardous substances in the study. Use water after its quality has been demonstrated to be favorable to the survival and reproduction of daphnia, from the same source as the water in which they were bred.

(ii) Use dechlorinated tap water, natural water supplies, or reconstituted water.

(iii) Aerate sufficiently prior to use, and prepare the temperature.

(6) Dissolved oxygen concentration

Maintain the dissolved oxygen concentration as at least 60% of the saturate concentration.

(7) pH

Do not adjust the pH of the test solution.

9. Observation and measurement

(1) Observation of the general condition of test organisms

Observe and record whether immobilization has occurred 24 and 48 hours after the initiation of exposure.

(2) Measuring of the test substance concentration

(i) When using TGAI as the test substance, measure the concentration of test substance in each test group at least at the initiation and end of exposure, and prior to and following water changes.

When setting the number of containers in each test group, take equal volumes of test solution from all containers, mix them, and use the mixture to measure of the test sample.

(ii) During the exposure period, the test substance concentration should be 80% or more than the setting concentration.

(3) Measurement of environmental conditions

(i) Confirm the quality of the dilution water prior to the study.

(ii) Measure water temperature, dissolved oxygen concentration, and pH of the test solution in each test group at least at the initiation and end of exposure, and prior to and following water changes.

10. Method of processing results

(1) Use an established method for caluculating EC_{50}, based on the immobilization rates results for each concentration.

(2) If the measured values for test substance concentration range ± 20% or more above the setting concentration, compute EC_{50} on the basis of the mean value of the measured concentrations.

11. Reporting

(1) The test substance

(2) The test organisms

Species name, history (source from which they were obtained, breeding method, etc.), EC_{50} of positive standard substance, etc.

(3) Test method

Exposure conditions, environmental conditions, items for observation and measurement, etc.

(4) Study results

(i) EC_{50}, and its 95% confidence limit (at the time of each observation, if possible)

(ii) Method of calculating EC_{50}

(iii) NOEC (If NOEC values have not been obtained, record the reason.)

(iv) The cumulative immobilization rate in each test group at the time of each observation

(v) A graph of the concentration/immobilization rates curve at the end of the exposure period

(vi) Observed effects

(vii) Measured values of test substance concentration (only when using TGAI as the test substance)

(viii) Results of measurement of environmental conditions

Water quality, dissolved oxygen concentration, pH, etc.

(ix) Other items

Test solution conditions, and other items that might affect study results.

12. Study validity

(1) The immobilization rate must not exceed 10% in control groups at the end of the exposure period.

(2) It is unacceptable for daphnia to be floating on the surface of the water in the control group at the initiation of exposure.

(3) The dissolved oxygen concentration must be maintained as at least 60% or more of the saturate concentration.

Daphnia reproduction toxicity studies (2-7-2-2)

1. Objective

The objective of these studies is to establish safe use methods of using agricultural chemicals by obtaining scientific information regarding effects of the test substances for the reproducibility on crustaceans.

2. Definitions

(1) Reproduction rate: This refers to the mean cumulative number of living offspring produced (surviving young daphnia) per parent.

(2) Median effect concentration (EC_{50}): The test substance concentration at which the reproduction rate of the test organisms is inhibited by 50% during the exposure period as compared to the control.

(3) Lowest observed effect concentration (LOEC): The lowest test concentration at which effects are observed, in terms of reproductivity, the mortality rate among parent daphnia, etc. as compared to the control.

(4) No observed effect concentration (NOEC): The highest test concentration at which no effects are observed, in terms of reproductivity, the mortality rate among parent daphnia, etc. as compared to the control.

(5) Test substance: TGAI used in studies.

(6) Semi-static test: A test that is conducted according to a system in which the test solution is changed in each container during each fixed period.

(7) Flow-through test: A test that is conducted according to a system in which test solution is supplied continuously.

3. Test organisms

(1) Organism species

(i) Use *Daphnia magna*. However, another species of *daphnia* may be used if equivalent study results can be obtained thereby.

(ii) Use test organisms of known history (the source from which they were obtained, methods of breeding, etc.).

(2) Life stage

Use individuals that are no more than 24 hours old (hereinafter referred to as "young daphnia").

(3) Breeding of parent daphnia

The parent daphnia used to obtain young daphnia should be kept for a fixed period under conditions as close to the study environmental conditions as possible (same water quality as that of the dilution water used in the studies, water temperature, etc.). Use daphnia that are healthy and in their reproductive prime (usually 2-4 weeks old).

4. Exposure method.

Conduct the studies under semi-static and flow-through conditions.

5. Exposure period

Conduct the studies for 21 days.

6. Number of test organisms and test groups

(1) Number of test organisms

Use at least 10 test organisms in each test group. Divide the organisms into numbers of individuals as necessary for observation.

(2) Design of test groups

(i) Test concentration groups

a. Set groups of at least 5 different concentrations in a geometrical series.

b. Establish test concentrations and a factor for concentration on the basis of preliminary study or acute immobilization study results.

c. It would be desirable to include within the concentration range the concentrations at which all test organisms are affected, and that at which there are no effects, as 1 level each, and at least 2 more levels at which some of the fish die.

(ii) Control groups

a. Establish as a control a group that is not treated with the test substance.

b. When using a solubilizing agent to prepare the test solution concentrate, establish a control group for a solubilizing agent, treated with the maximum concentration of solubilizing agent to be used in the study.

7. Preparation the test solution

The method of preparing the test solution is as described below.

(1) Usually, dilute test solution concentrate of a high concentration to obtain test solution. Use a solubilizing agent, such as organic vehicle, emulsifier, or dispersing agent, if necessary for precise preparement of test solution concentrate, but it is desirable to avoid using auxiliary agents insofar as possible.

(2) Use a solubilizing agent that is of low toxicity to the organisms, which has not been noted to have adverse effects on the organisms at the concentration used in the study, and which does not change the properties of the test substance.

(3) The auxiliary agent concentration in the test solution should not exceed 100 mg/l (or 0.1 ml/l).

8. Environmental conditions

(1) Amount of test solution

At least 50 ml per individual daphnia.

(2) Water temperature

It is desirable to set the temperature in the range of 18-22° C, and allow it to vary within a range of ± 1° C.

(3) Light

It is desirable to light for 16 hours.

(4) Feeding

Feed the daphnia unicellular green algae, such as chlorella.

(5) Dilution water

(i) Do not use water that contains hazardous substances in the study. Use water after its quality has been demonstrated to be favorable to the survival and reproduction of daphnia, from the same source as the water in which they were raised.

(ii) Use dechlorinated tap water, natural water supplies, or reconstituted water.

(iii) Expose to air sufficiently prior to use, and prepare the temperature.

(6) Dissolved oxygen concentration

Maintain a dissolved oxygen concentration of at least 3 mg/L during the exposure period.

(7) pH

Do not adjust the pH of the test solution.

9. Observation and measurement

(1) Observation of the general condition of test organisms

Count the number of living and dead parent daphnia, as well as the number of surviving offspring. Regularly observe and record the condition of parent daphnia, as well as whether or not dead young daphnia, aborted eggs, or resting eggs are present.

(2) Measuring test substance concentration

(i) Measure the test substance concentration in each test concentration group.

(ii) During the exposure period, the test substance concentration should be 80% or more than the nominal concentration.

(3) Measurement of environmental conditions

(i) Confirm the quality of the dilution water prior to the study.

(ii) Measure water temperature, dissolved oxygen concentration, hardness, and pH of the test solution in each test group.

10. Method of processing results

(1) EC_{50} and 95% confidence limit

Calculate EC_{50} according to the common method, in so far as possible, using the mean total number of living offspring produced per parent (surviving young *daphnia*) in each control group (or auxiliary agent control group) and each test concentration group.

(2) LOEC and NOEC

Calculate the total cumulative number of living offspring produced per parent (surviving young *daphnia*) in each test container, and determine by statistical means whether there are significant differences between the control group (or solubilizing agent control group) and each test concentration group. Determine the lowest concentration(LOEC) at which significant differences are noted and the maximum concentration(NOEC) at which significant differences are not observed, in comparison with the control group (or solubilizing agent control group).

(3) If the measured values for test substance concentration vary ± 20% or more from the nominal concentration, compute EC_{50} on the basis of the mean measured concentration.

11. Reporting

(1) The test substance
(2) The test organisms
Species name, history (source from which they were obtained, method of care, etc.), etc.
(3) Test method
Exposure conditions, environmental conditions, items for observation and measurement, etc.
(4) Study results
(i) Mortality rate of parent daphnia
(ii) Reproductive rate
(iii) EC_{50}, and its 95% confidence limit (if possible)
(iv) Calculating method of EC_{50}
(v) LOEC and NOEC (If these have not been obtained, record the reason.)
(vi) Calculating method of LOEC and NOEC
(vii) The mortality rate and reproductive rate among parent daphnia in each test group at the time of each observation
(viii) Graph of the concentration/parent daphnia mortality rate curve at the end of the exposure period
(ix) Observed effects
(x) Days until the first offspring appear in each test concentration group
(xi) Measured values of test substance
(xii) Results of measurement of environmental conditions
Water quality, dissolved oxygen concentration, hardness, pH, etc.
(xiii) Other items
Test solution conditions, and other items that might affect study results.

12. Study validity
(1) The mortality rate of parent daphnia must not exceed 20% in control groups at the end of the exposure period.
(2) The mean total cumulative number of living offspring produced per parent in the control group must be at least 60 individuals.

Algae growth inhibition studies (2-7-3)

1. Objective
The objective of these studies is to establish safe use methods of handling agricultural chemicals by obtaining scientific information regarding the effects of the test chemical on algae growth.

2. Definitions
(1) Cell concentration: The number of cells per ml.
(2) Growth: The increase in cell concentration during the test period.
(3) Growth rate: The increase in cell concentration per unit of time.
(4) Median effect concentration (EC_{50}): The test substance concentration at which growth is inhibited by 50% as compared with the control group.
(5) No observed effect concentration (NOEC): The maximum concentration at which no effects are observed as compared to the control group.
(6) Test substance: TGAI or formulation of the agricultural chemical to be tested.
(7) Standard substance: Substance used for confirming the reproducibility of test conditions.
(8) Test chemical: Test substance and standard substance used in the studies.

3. Test organisms
(1) Organism species
(i) It is desirable to use *Selenastrum capricornutum*. However, the species and strains listed below, as well as others,

may be used if they are more convenient for culturing and testing, and if they grow quickly.

a. *Selenastrum capricornutum* (ATCC 22662 strain)

b. *Scenedesmus subspicatus* (86.81 SAG strain)

c. *Chlorella vulgaris* (CCAP 211/11b strain)

(ii) It would be desirable to confirm EC_{50} with the standard substance.

(2) Culturing method

Algae should be cultivated under conditions similar to the study conditions. Use algae in its logarithmic growth phase. In principle, culture under sterile conditions.

(3) Initial cell concentration

An initial cell concentration of approximately 10^4 cells/ml in the culture medium is appropriate.

4. Exposure method

Use a method whereby algae is exposed in the culture medium with test substance. Use either shaken or static culturing.

5. Exposure period

Set a 72-hour period. However, this may be extended to 96 hours.

6. Establishing test groups

(1) Establishing test substance concentration groups

(i) Establish at least 5 different concentration groups in geometric series.

(ii) Determine test concentrations and their factor on the basis of preliminary studies.

(iii) It would be desirable to include within the concentration range the concentration at which test algae growth is almost entirely inhibited, and that at which there is no inhibition, as 1 level each, and at least 2 more levels at which algae growth is partially inhibited.

(2) Establishing control groups

(i) Establish control groups that are not treated with the test substance.

(ii) When using a solubilizing agent to prepare the test culture medium, establish a solubilizing agent control group, treated with the maximum concentration of solubilizing agent to be used in the study.

(3) Replication of test group groups

Conduct the test 3 times in each test concentration and control group.

7. Preparation method of test culture medium

The test culture medium is prepared by a method as described below. Note that it is desirable to prepare test culture medium immediately prior to use in the study.

(1) When using TGAI as the test substance

(i) When using a water soluble TGAI, dissolve the test substance in culture medium that has been properly sterilized, and prepare it as the test stock solution. After the test stock solution has been diluted with sterilized culture medium, prepare test culture medium by adding the algae suspension, and prepare.

(ii) When using a TGAI that is not water soluble, prepare the test culture medium according to either of the following methods.

a. Prepare the test culture medium, using test stock solution consisting of test substance dissolved in a solubilizing agent, such as organic vehicle. Use a solubilizing agent that is of low toxicity to test organisms, which has not been noted to have adverse effects on test organisms at the concentration used in the study, and which does not change the properties of test substance.

The solubilizing agent concentration in the test solution should not exceed 100 mg/l (or 0.1 ml/l).

b. Add the amount of test substance necessary for each concentration to the sterilized culture medium, by sterilized conditions. After stirring or ultrasonic treatment etc., prepare the test culture medium by adding algae suspension.

(2) When using formulation as a test substance

Prepare test stock solution by adding formulation to sterilized culture medium and stirring. After the test stock solution has been diluted with culture medium, prepare test culture medium by adding the algae suspension, and prepare. Do not use a solubilizing agent with the formulation.

8. Environmental conditions

(1) Culturing method

(i) Culturing under sterilizing conditions.

(ii) Maintain the test culture medium as a suspension throughout the test period; shake or stir the test containers, in order to promote ventilation. When conducting static culturing, shake at least twice per day.

(2) Culture temperature

Set the temperature at 21-25° C, and allow it to vary within a range of ± 2 ° C during the test period.

(3) Light

It would be desirable provide continuous, uniform light within a spectra range of 400-700 nm, with a light source of 4000 lux at the surface level of the solution.

(4) Culture medium

(i) Types of media

It is desirable to use either OECD medium (OECD Test Guideline 201: Alga, Growth Inhibition Test (1984)) or AAP (AGP) medium (US EPA: Alga Assay Procedure: Bottle Test, National Environmental Research Center, Corvallis, Oregon (1971)).

(ii) Amount of culture medium

The amount of culture medium may vary according to the measuring method of cell concentration, and according to the determing method of determining test substance concentration, but should be on about100 ml.

9. Observation and measurement

(1) Measurement of cell concentration

Measure cell concentrations in each test containers every 24 hours following the initiation of exposure, until the end of exposure.

(2) Measurement of test substance concentration

(i) If stock solution has been used as the test substance, measure the concentration of test substance in each test concentration group at least at the beginning and end of exposure.

(ii) Collect test solution from each container in each test substance concentration group, combine the solution thus collected, and use it as a sample for measurement.

(3) Measurement of environmental conditions

(i) Measure test water temperature and pH readings of test culture medium from 1 container in each test group (test concentration and control groups).

(ii) Measure at least at the beginning and end of exposure.

10. Processing methods of results

(1) Methods of calculating concentration-percentage inhibition rate of cell growth

Tabulate the cell concentration in each test concentration and control group, together with the measurement time, and the test substance concentration(Actual measured concentration, if TGAI is used as test substance). Draw a growth curve by plotting the mean number of cells in each test concentration group and control group versus time. Calculate the growth inhibition rate for each concentration by comparing areas under the growth curves or by comparing growth rates.

(2) Calculating EC_{50}

Use an established method for calculateing EC_{50}, based on the growth inhibition rate results for each concentration.

11. Conduct of recovery study

If necessary, conduct of recovery studies in order the degree to which algae concentration recovers by diluting culture medium solution confirmed to have inhibited growth and culturing again.

12. Reporting

(1) The test substance

(2) The test organisms

Species name, strain name, EC_{50} of standard substance, etc.

(3) Test method

Exposure conditions, environmental conditions, items for observation and measurement, etc.

(4) Study results

(i) EC_{50}, and its 95% confidence limit (at the time of each observation, if possible)

(ii) Method of calculating EC_{50}

(iii) NOEC (If NOEC values have not been obtained, record the reason.)

(iv) Cell concentration and the average in each test group at the time of each observation

(v) Method of counting cells

(vi) Growth curve

(vii) A graph showing the concentration/growth inhibition rate relationship

(viii) Observed effects

(ix) Measured values of test substance concentration (only when using TGAI as the test substance)

(x) Measurement results of environmental conditions

Water quality, pH, etc.

(xi) Other items

Test solution conditions, and other items that might affect study results

13. Study validity

At 72 hours after the initiation of the study, the cell concentration in the control group must have increased by at least 16 times the initial cell concentration.

Studies of effect on beneficial organisms other than aquatic organisms(2-8-1~4)

Bee toxicity studies (2-8-1)

1. Objective

The objective of these studies is to establish safe use methods of handling agricultural chemicals by obtaining scientific information regarding the influence of the test substance on bees.

2. Study method

(1) Conduct acute toxicity studies (acute oral toxicity studies or contact toxicity studies). However, there is no objection to conduct of studies according to other methods, if they are scientifically valid.

(2) If severe toxicity is noted in acute toxicity study results, field toxicity studies should be conducted.

Silkworm toxicity studies (2-8-2)

1. Objective

The objective of these studies is to establish safe use methods of handling agricultural chemicals by obtaining scientific information regarding the influence of the test substance on silkworms.

2. Study method

(1) Conduct acute oral toxicity studies. However, there is no objection to conduct of studies according to other methods, if they are scientifically valid.
(2) If severe toxicity is noted in acute oral toxicity study results, residual toxicity studies should be conducted.

Toxicity studies on natural enemy insects, etc. (2-8-3)

1. Objective

The objective of these studies is to establish safe use methods of handling agricultural chemicals by obtaining scientific information regarding the influence of the test substance on insects other than the target insects, such as natural enemy insects.

2. Study method

(1) Conduct acute toxicity studies. However, there is no objection to performance of studies according to other methods, if they are scientifically valid.
(2) If severe toxicity is noted in acute toxicity study results, field effect studies should be conducted.

Avian effect studies (2-8-4-1, 2)

Avian acute oral toxicity studies (2-8-4-1)

1. Objective

The objective of these studies is to establish safe use methods of handling agricultural chemicals by obtaining scientific information regarding the effects of a single oral administration of the test substance on birds.

2. Study method

(1) No particular guidelines. Conduct by a scientifically valid method.
(2) For example, the US EPA document, "Ecological Effects Test Guidelines OPPTS 850.2100 Avian Acute Oral Toxicity Test — Public Draft (712-C-96-139, April, 1996)" is available as a study method.

Avian dietary toxicity studies (2-8-4-2)

1. Objective

The objective of these studies is to establish safe methods of handling agricultural chemicals by obtaining scientific information regarding the effects of the test chemical on birds when it is administered to them via their feed, as a more realistic exposure route.

2. Study method

(1) No particular rules. Conduct a scientifically valid method.
(2) For example, the OECD Test Guideline 205, entitled "Avian Dietary Toxicity Test (1984)" is available as a study method.

Studies of the properties, stability, degradability, etc. of active ingredients (2-9-1~16)

1. Objective

The objective of these studies is to obtain basic scientific information such as their properties, stability and degradability that is indispensable for evaluating aspects of the safety of agricultural chemicals.

2. Specific study content, etc.

This set of studies is comprised of the following (2-9-1~16).

(1) Color studies (2-9-1)

(i) Test method

Color should be observed visually at room temperature and ordinary pressure.

(ii) Items to be reported

Color (Record as "white", "light yellow", "brown", etc.)

(2) Studies of ohisical form of the substance (2-9-2)

(i) Test method

Physical form should be observed visually at room temperature and ordinary pressure.

(ii) Items to be reported

Physical form (Record as "solid (crystal)", "solid (powder)", "liquid", "gas", etc.)

(3) Odor studies (2-9-3)

(i) Test method

Odor should be observed organoleptically at room temperature and ordinary pressure.

(ii) Items to be reported

Odor (Record as "pungent odor", "aromatic odor", etc.)

(4) Spectrum studies (2-9-4)

(i) Test method

a. Ultraviolet-visible absorption (UV/VIS) spectrum should be measured in accordance with OECD Guideline 101 (adopted May 12, 1981).

b.Infrared (IR) spectrum, nuclear magnetic resonance (NMR) spectrum (^{1}H, ^{13}C, etc.), and mass spectrum (MS) should be observed , using appropriate measuring devices.

(ii) Items to be reported

Measurement conditions and chart. For UV/VIS, record absorption wavelength (nm) and molar extinction coefficient; for IR, record absorption wavelength (cm^{-1}); for NMR, record ppm; for MS, record measured values as m/z. Insofar as possible, clarify the assignment of each NMR and MS peak with relevant structural formula.

(5) Melting point studies (2-9-5)

(i) Test Method

Melting point should be measured in accordance with OECD Test Guideline 102 (adopted July 27, 1995).

(ii) Items to be reported

Melting point (° C)

(6) Boiling point studies (2-9-6)

(i) Test Method

Boiling point should be measured in accordance with OECD Test Guideline 103 (adopted July 27, 1995).

When boiling does not occur at normal pressure, take readings under reduced pressure conditions.

(ii) Items to be reported

Boiling point (° C). Record observed decomposition, if any.

(7) Vapor pressure studies (2-9-7)

(i) Test Method

Vapor pressure should be measured in accordance with OECD Test Guideline 104 (adopted July 27, 1995).

(ii) Items to be reported

a. Vapor pressure (Pa units)

b. Test temperature (° C)

(8) Studies of solubility in water (2-9-8)

(i) Test Method

Solubility in water should be measured in accordance with OECD Test Guideline 105 (adopted July 27, 1995).

(ii) Items to be reported

a. Solubility in water (mg/l or g/l)

b. Test temperature (° C)

(9) Studies of solubility in organic solvent (2-9-9)

(i) Test Method

a. Solubility in organic solvent should be measured in accordance with OECD Test Guideline 105 (adopted July 27, 1995).

b. As an organic solvent, use a nonpolar hydrocarbon (such as hexane or heptane), an aromatic hydrocarbon (such as xylene or toluene), a halogenated hydrocarbon (such as dichloromethane), a ketone (such as acetone), an alcohol (such as methanol or ethanol), or an ester (such as ethyl acetate).

(ii) Items to be reported

a. Solubility in organic solvent (mg/l or g/l)

b.Test temperature (° C)

(10) Soil adsorption studies (2-9-10)

(i) Test Method

The study should be conducted in accordance with OECD Test Guideline 106 (adopted January 21, 2000). However, in general more than one soil should be selected among each Types 2, 3, 4 and 5 of the 7 soil types indicated in said guideline and at least one must be volcanic ash soil. Soil equilibrium should be measured at 25° C.

(ii) Items to be reported

a. Soil adsorption coefficient (in units of K^{ads}_{F} and $K^{ads}_{F\,oc}$)

b. Test temperature (° C)

(11) n-octanol/water partition coefficient studies (2-9-11)

(i) Test Method

The test should be conducted in accordance with OECD Test Guideline 107 (adopted July 27, 1995) or 117 (adopted March 30, 1989) at a temperature of 25° C.

(ii) Items to be reported

a. Octanol/water partition coefficient ($\log_{10}$ values)

b. Test temperature (° C)

(12) Density studies (2-9-12)

(i) Test Method

Density should be measured in accordance with OECD Test Guideline 109 (adopted July 27, 1995).

(ii) Items to be reported

a. Density (g/cm^3)

b. Test temperature (° C)

(13) Hydrolysis studies (2-9-13)

(i) Test Method

The test should be conducted in accordance with OECD Test Guideline 111 (adopted May 12, 1981).

(ii) Items to be reported

a. Estimated half-life for each pH level

b. Test temperature (° C)

(14) Dissociation constant study (2-9-14)

(i) Test Method

Dissociation constant should be determined in accordance with OECD Test Guideline 112 (adopted May 12, 1981).

(ii) Items to be reported

a. Dissociation constant (pKa)

b. Test temperature (° C)

(15) Thermal stability study (2-9-15)

(i) Test Method

The test should be conducted in accordance with OECD Test Guideline 113 (adopted May 12, 1981; excluding accelerated storage studies).

(ii) Items to be reported

Whether or not quality is changed due to heat, and the temperature at which deterioration occurs (° C)

(16) Photolysis study in water (2-9-16)

(i) Test method

Prepare a dilute solution, at a concentration of aqueous solubility or lower, and within a range enabling analysis, by adding the test substance to sterile distilled water. Determine the test substance's photolysis in water, using an artificial light source (one that has a wavelength distribution and light intensity similar to those of natural light). In such cases, conduct the studies at a water temperature of 25° C. Use quartz glass containers. Conduct control studies without light.

(ii) Items to be reported

a. Estimated half-life

b. Test temperature (° C), light intensity (w/m^2), and measured wavelength range (nm)

Studies of water polluting properties (2-10-1)

1. Objective

The objective of this study is to obtain scientific information regarding pollution of paddy water by agricultural chemicals used in paddies.

2. Design test plots (test paddies)

Design test plots treated and untreated with the test substance, as follows.

(1) Treatment of test plots (test paddies)

(i) In general, use concrete container of at least 1 m² (1 m x 1 m), enabling the amount of leaching water to be adjusted.

(ii) Use paddy soil, comprising gray lowland soil, gley soil, high-humidity andosol, or brown lowland soil for package. In general, use soil after pulverizing the soil without air drying, then selectively removing small stones and bulky organic matter, and mixing well, pack it with water into a soil layer of approximately 50 cm deep, while sufficiently de-aerating it.

(iii) Establish the plots so as to sufficiently reflect atmospheric and other environmental conditions in fields.

(iv) It would be desirable to set up a roof so that rainwater cannot enter, in order to prevent sudden increases, etc. in the amount of paddy water due to rainfall. In such cases, be sure that air flow is not impeded; and use materials with good light transmittance for the roof.

(2) Test plot (test paddy) control

(i) Allow approximately 1-2 cm of leaching per day throughout the study period, and maintain the plots in a flooded state with a water depth of approximately 5 cm. Eliminate drainage and runoff.

(ii) The water used should not contain substances that are likely to affect decomposition or interrupt analysis of the test substance.

(3) Crops cultivated in test plots

The crops cultivated in test plots should be the same one in application for registration, and in general should be cultivated in the conventional way.

3. Handling and treatment of the test substance

(1) The test substance should be used soon after it is prepared.

(2) The test substance should be stored under appropriate conditions. If the substance is to be stored for a long period after opening, its stability during the storage period should be confirmed.

(3) Apply the test substance once properly, in a formulation and a method (in terms of time, quantity, etc.) that is

relevant to the application for registration, and use the tools customarily used.

However, if it is difficult to use conventional tools in test paddies, other equivalent methods may be employed.

(4) Do not apply the test substance during rainy weather, or when rainfall is expected shortly after application. Unless rain will not affect results, e.g. the system is under a roof.

4. Sampling (paddy water)

(1) Method of sampling

(i) Collect samples in glass bottles, using a syringe and taking care not to mix in bulky organic matter and soil particles. Mix well.

(ii) Collect samples from at least 4 different places at a time (with, in general, water depth 2-3 cm).

(2) Sampling intervals and frequency

(i) In general, collect samples immediately before and after application (1-3 hours afterwards), as well as 1 day, 3 days, 7 days, and 14 days after application.

(ii) When there is a possibility that substances targeted for analysis may be detected in paddy water more than 14 days after application, continue collecting samples until the concentration detected is at least 1/10 of the maximum detected.

(iii) If set water outlet closing period is established in the method of usage relevant to the application for registration, water samples should also be collected on the last day period.

5. Handling of samples

(1) Transporting samples

(i) Care should be taken in transporting samples not to let the samples deteriorate or become contaminated. They should be transported rapidly, at low temperatures, but not frozen.

(ii) Handle samples properly, by affixing identifying labels, etc., in order to prevent confusion of samples during transport.

(2) Handling samples after transportation

Immediately upon receiving samples, verify their authenticity according to their identification labels, etc. Handle them properly, so as to avoid confusion among samples, and use them promptly for analyses.

6. Analysis of samples

(1) Target substances for analysis

The substances to be analyzed are the active ingredients of agricultural chemicals related to the test substance, as well as substances formed biologically and chemically. However, this does not apply to substances detected in extremely minor quantities in water, or which have been deemed non-harmful due to their extremely low toxicity, etc.

(2) Method of analysis

(i) Adopt a method by which the target substance can be accurately analyzed.

(ii) Express the amount of target substance residue as mg/l.

(iii) Analyze each sample at least twice, and use the mean value from these analyses as the measured value.

(iv) Confirm the precision of the analytical method by relative standard deviation(RSD) of recovery study within the range of concentrations in which detection of the target substances is anticipated.

(v) The sensitivity of the analytical method is expressed as the limit of quantification, that is, the minimum concentration at which a sufficient recovery rate can be obtained in all operations for analysis of samples.

(vi) Confirm the recovery rate of the analytical method, at the limit of quantification and at the range of concentrations in which detection of the target substances is anticipated, using samples that have been collected from untreated plots, and to which a known quantity of the target substance has been added.

(vii) In general, analyze the samples for analysis promptly after the collection. If tentative storage of samples is unavoidable, store them under appropriate conditions, conduct storage stability examinations to determine the stability of target substances during the storage period.

(viii) Conduct storage stability examinations of stored samples, using samples that have been collected from

untreated plots, and to which a known quantity of the target substance has been added and stored, under the same conditions, for at least the same period, and analyze according to the same methods by which test samples are analyzed.

7. Items to be reported
(1) Institution preparing study results (field test facilities and institution conducting analyses)
(2) Test substance
(3) Test conditions
(4) Method of analysis (summary and details)
(5) Limit of quantification and recovery rate for each target substance
(6) Details regarding sample preparation
(7) Results of analysis (analytical values at each time of sample collection)

< Studies of residue in crops, etc.>

Studies of residue in crops, etc.

Studies of residue in crops (3-1-1)

1. Objective

The objective of these studies is to obtain scientific information regarding the degree of residue of agricultural chemicals in crops.

2. Test crops
(1) Choose representative varieties and cropping types from among crops relevant to the application for registration.
(2) Select a standard method of cultivation. Base the method on greenhouse cultivation or cultivation without bags, since cultivation conditions (greenhouse versus outdoor, with bags versus without bags) have an especially large effect on residue.

3. Establishing test plots (fields)
(1) Establish plots treated and untreated with the test substance as test plots.
(2) The test plots must be of sufficient area to ensure a sufficient quantity of crops for performance of analyses.
(3) Measures must be devised for preventing contamination of the test plots with scattered agricultural chemicals from elsewhere.
(4) For each field, establish the number of days during which observations of fate of residue of the test substance are made, in principle, in each test substance treatment plot. However, these limitations do not apply to test substances that are used in the initial growth stage of crops, or to those used on limited occasions, such as soil treatment chemicals.

4. Cultivation of test crops
(1) Conduct cultivation management by the ordinary methods, such that the test crops will be in a marketable condition at harvest time.
(2) If extermination of harmful insects, etc. is unavoidable, choose a method that will not affect study results.

5. Handling and preparation of the test substance
(1) The test substance should be employed soon after it is prepared.
(2) The test substance should be stored under appropriate conditions. If the substance is to be stored for a long period, after being opened, its stability during the storage period should be confirmed.
(3) Apply the test substance once properly, in a formulation and according to a method of usage (in terms of time,

number of applications, quantity, etc.) that is relevant to the application for registration, and using the tools customarily employed. However, if it is difficult to use said tools in test paddies, other equivalent methods may be substituted.

(4) Do not apply the test substance during rainy weather, or when rainfall is expected shortly after application. However, when rain will not affect results, due to construction of a roof, this limitation is unnecessary.

6. Test samples

(1) Sampling portions and sampling volume should be in accordance with Matter No. 1a in the standards stipulated by the Director-General of the Environment Agency (Environmental Agency Bulletin No. 46, July 24, 1973), establishing whether or not Article 3, Paragraph 1, Numbers 4 to 7, of the Agricultural Chemicals Regulation Law applies. For products for which it is difficult to meet the standards of sampling volume, the volume can be appropriately altered in consideration of a variation within the same sample and precision of analysis.

(2) Use a proper method of collecting samples, so that there is no bias in sampling.

(3) Samples should be in a marketable condition and should also be, insofar as possible, of uniform size (in terms of length and thickness). Do not collect defective crops (immature crops, as well as those injured by disease, insects, or chemicals).

(4) Use proper methods of collecting and packaging samples so as to avoid confusion among samples, as well as contamination.

7. Handling of samples

(1) Transporting samples

(i) Care should be taken in transporting samples so that they do not deteriorate or become contaminated. They should be transported rapidly, at low temperatures, but not frozen; this does not apply to sample that have been sun-dried or mechanically dried.

(ii) Handle samples properly, by affixing identifying labels, etc., in order to prevent confusion among samples during transport.

(2) Handling samples after transportation

Immediately upon receiving samples, verify their authenticity according to their identification labels, etc. Handle them properly, so as to avoid confusion among samples, and use them promptly for analyses.

8. Analysis of samples

(1) Target substances for analysis

The substances to be analyzed are the active ingredients of agricultural chemicals related to the test substance, as well as substances generated in the course of the biological and chemical changes they undergo (hereinafter referred to as "component substances, etc."). However, this does not apply to substances that leave extremely minimal residues, and which have been deemed non-harmful due to their extremely low toxicity, etc.

In general, analyze the component substances, etc. of spreading agent as well as the agricultural chemicals to which they are applied. However, spreading agents may be listed in a following table for any valid reason, based on such factors as their effect on residue of the substances to which they are applied.

(2) Analysis portions

Analysis portions should be in accordance with rules and standards pertaining to food and food additives (Ministry of Health and Welfare Bulletin No. 370, December 28, 1959), and with Matter No. 1a in the standards stipulated by the Director-General of the Environment Agency (Environmental Agency Bulletin No. 46, July 24, 1973, establishing whether or not Article 3, Paragraph 1, Numbers 4 to 7, of the Agricultural Chemicals Regulation Law applies. As regards rice plants, the rice straw should be analyzed as well.

(3) Method of analysis

(i) Use all or uniform portions of samples from each analysis portions, after they have been crushed.

(ii) Adopt a method by which the target substance can be accurately analyzed. However, when an analytical method has been stipulated upon establishment of food regulations (standards for residual agricultural chemicals) or standards for withholding registration of agricultural chemicals, use the method so stipulated.

(iii) Express the amount of target substance residue as ppm.

(iv) Analyze each sample at least twice.

(v) Confirm the precision of the analytical method by means of the coefficient of variation within the range of concentrations in which detection of the target substances is anticipated.

(vi) The sensitivity of the analytical method should be consistent with study objectives, and should be expressed according to the limit of determination, that is, the minimum concentration at which a recovery rate can be obtained that is sufficient for all operations involved in analysis of samples.

(vii) Confirm the recovery rate of the analytical method, within the limit of quantification and the range of concentrations in which detection of the target substances is anticipated, using samples that have been collected from untreated plots, and to which a known quantity of the target substance has been added.

(viii) In general, use samples for analysis promptly after they have been collected. If temporary storage of samples is unavoidable, store them under appropriate conditions, conduct storage stability examinations to determine the stability of target substances during the storage period.

(ix)Conduct storage stability examinations of stored samples, using samples that have been collected from untreated plots, and to which a known quantity of the target substance has been added, under the same conditions, for at least the same period, and according to the same methods by which test samples are analyzed.

9. Items to be reported

(1) Institution preparing study results (field test facilities and institution conducting analyses)

(2) Test substance

(3) Methods of test crop cultivation, test substance use, etc.

(4) Weather conditions during the cultivation period (air temperature, rainfall, sunshine, etc.)

(5) Target substance for analysis

(6) Method of analysis (summary and details)

(7) Limit of quantification and recovery rate in each analysis

(8) Details regarding sample preparation

(9) Results of analysis

Table

	Effect of the relevant spreading agent on the residue of the agricultural chemical to which it is applied	
	When it is confirmed that there is no likelihood that it increases the residue of the agricultural chemical to which it is applied	When it is confirmed that there is a likelihood that it increases the residue of the agricultural chemical to which it is applied
1. Safety confirmed, for the reason that the toxicity of the component substances of the relevant spreading agent are of extremely low toxicity, etc.		Component substances, etc. of the agricultural chemical to which the spreading agent is to be applied
2. Safety confirmed for the reason that there is no likelihood that humans would ingest the component substances of the relevant spreading agent over the long term; or because, if they did ingest it, the amount consumed would be extremely minute.		Component substances, etc. of the agricultural chemical to which the spreading agent is to be applied
3. Cases to which neither 1 nor 2 above applies.	Component substances, etc. of the spreading agent	Component substances, etc. of the spreading agent and the agricultural chemical to which it is applied

Studies of translocation to milk (3-1-2)

1. Objective

These studies are conducted when agricultural chemicals remain in rice straw and crops used as animal feed, and the residues detected are at or above a certain concentration, in studies of residue in crops. Their objective is to obtain information regarding the degree of translocation to milk.

2. Test animals

In general, use at least 2 dairy cows with daily milk yields of at least 15 kg each.

3. Care conditions

According to the methods of care at the relevant facilities.

4. Method of administration and dosage

Administer to each cow, orally, once per day (immediately after morning milking) for 7 consecutive days, in rice straw obtained in studies of residue in crops (or in other crops used as feed), twice the maximum residual of the test substance permitted under existing regulations.

However, there is no objection to forcible oral administration by capsule.

5. Collecting samples

(1) Collect samples prior to administration, on the 1st, 3rd, and 7th days of administration, as well as on the 1st, 3rd, and 5th days following the end of administration. Milk the cows in the morning and evening, and collect 100 ml from each milking as a sample.

(2) Place the samples in separate containers according to collection time; freeze them, and use them for analysis.

6. Storage, transportation, and analysis of samples

(1) Conduct storage, transportation, and analysis as in studies of residue in crops.

(2) Pool the samples collected in the mornings and evenings for use in analyses.

7. Items to be observed

(1) General symptoms

Observe daily the activity, food and water intake, feces, etc. of the test animals, and keep records.

(2) Body weight
Weigh the animals at the commencement of the study.
(3) Lactation
Take daily readings during the study period.

8. Reporting results
Follow the guidelines for studies of residue in crops.

Soil residue studies (3-2-1, 2)

Studies in containers (3-2-1-1)

1. Objective
The objective of these studies is to obtain scientific information regarding the degree of residue of agricultural chemicals in soil in containers.

2. Test soil
Use in studies soil that has not received scatterings of agricultural chemicals that might hinder analyses in these studies. Select soils that have been collected domestically, and are of at least 2 types with different properties in terms of soil texture, parent material, and other aspects. Among these, at least 1 type should in general be field soil used in field studies.
Use paddy soil for studies of substances that are to be used in paddies, and use upland soil for studies of substances that are to be used in upland fields.

3. Test soil preparation
(1) After crushing and passing through a 5 mm sieve test soil that has not been air dried, fill at least 2 runs of glass test containers with soil to a soil depth of at least 1 cm.
(2) In general, the moisture content should be as follows during the test period.
(i) For upland field soil, maintain the moisture content within a range of 50-60% of the soil's water holding capacity, using distilled water.
(ii) For paddy soil, maintain flooded conditions, with a water depth of at least 1 cm above the surface of the soil, using distilled water.
(3) Maintain a fixed soil temperature (± 2° C), within a range of 25-30° C, throughout the study period.
(4) Test soil should be used in preliminary cultivation for at least 1 week after having been placed in test containers.

4. Handling of test substance
(1) Apply the test substance once, dissolved in water or a small amount of organic solvent (acetone, etc.). Stir or shake the soil after application, to disperse the test substance uniformly.
(2) In general, the amount of the test substance used in a single field application should be equal to the amount required to disperse the agricultural chemical associated with the test substance evenly in soil to a depth 10 cm below the surface in a concentration suitable for the relevant studies.

5. Collecting samples
(1) Collection schedule and the number of times collected
Collection samples once each immediately prior to and following addition of the test substance, and at least 4 times thereafter.
(2) Method of collection
At each collection time, collect all of the soil in a container. In the case of flooded samples, collect the water in the container together with the soil.

6. Handling of samples

(1) Transporting samples

(i) Care should be taken in transporting samples so that they do not deteriorate or become contaminated. They should be transported rapidly, at low temperatures, but not frozen.

(ii) Handle samples properly, by affixing identifying labels, etc., in order to prevent confusion among samples during transport.

(2) Handling samples after transportation

Immediately upon receiving samples, verify their authenticity according to their identification labels, etc. Handle them properly, so as to avoid confusion among samples, and use them promptly for analyses.

7. Study period

In general, set the study period as the time until the analytical values of the target substances (when there are multiple target substances, compute the total amount of active ingredient, based on the analytical values) in test soil decline to approximately 10% of the concentration immediately following addition (if the values do not decline to approximately 10%, the time until they decline to less than 1/2 of the concentration immediately following addition).

8. Analysis of samples

(1) Target substances for analysis

The substances to be analyzed are the active ingredients of agricultural chemicals related to the test substance, as well as substances generated in the course of the biological and chemical changes they undergo. However, this does not apply to substances that leave extremely minute residues, and which have been deemed non-harmful due to their extremely low toxicity, etc.

(2) Method of analysis

(i) Adopt a method by which the target substance can be accurately analyzed.

(ii) Express the amount of target substance residue as concentration in dry soil (mg/kg).

(iii) Analyze each sample at least twice, and use the mean value from these analyses as the measured value.

(iv) Confirm the precision of the analytical method by means of the coefficient of variation within the range of concentrations in which detection of the target substances is anticipated.

(v) The sensitivity of the analytical method should be consistent with study objectives, and should be expressed according to the limit of quantification, that is, the minimum concentration at which a recovery rate can be obtained that is sufficient for all operations involved in analysis of samples.

(vi) Confirm the recovery rate of the analytical method, within the limit of quantification and the range of concentrations in which detection of the target substances is anticipated, using samples that have been collected from untreated plots, and to which a known quantity of the target substance has been added.

(vii) In general, use samples for analysis promptly after they have been collected. If temporary storage of samples is unavoidable, store them under appropriate conditions, conduct storage stability examinations to determine the stability of target substances during the storage period.

(viii) Conduct storage stability examinations of stored samples, using samples that have been collected from untreated plots, and to which a known quantity of the target substance has been added, under the same conditions, for at least the same period, and according to the same methods by which test samples are analyzed.

9. Items to be reported

(1) Institution preparing study results (field test facilities and institution conducting analyses)

(2) Test substance

(3) Study conditions

(4) Target substances for analysis

(5) Method of analysis (summary and details)

(6) Limit of quantification and recovery rate in each analysis

(7) Method of preparing samples, etc.

(8) Results of analysis

Results should include the analytical values at each sample collection time, decay curve, the time until the concentration declines to less than 1/2 of its maximum value. If multiple components are targeted for analysis, compute the total amount of all active ingredients, and report the time until the concentration for this total amount decline to less than 1/2 of its maximum value.

Field studies (3-2-1-2)

1. Objective

The objective of these studies is to obtain scientific information regarding the degree of residue of agricultural chemicals in soil under field conditions.

2. Test soil

(1) When the test fields are domestic and include soil from at least 2 locations with different properties in terms of soil texture, parent material, and other aspects, select locations that are not contaminated by agricultural chemicals, etc. that might inhibit analyses.

(2) If fields with differing soil properties cannot be used, due to unavoidable circumstances, fields in which differing conditions other than soil properties (such as weather conditions) prevail may be used. Use paddies for studies of substances that are to be used in paddies, and use upland fields for studies of substances that are to be used in upland fields, so that studies are conducted under conditions in which the representative crops, on which the relevant agricultural chemical is to be used, would be cultivated.

3. Handling and preparation of the test substance

(1) The test substance should be employed soon after it is prepared.

(2) The test substance should be stored under appropriate conditions. If the substance is to be stored for a long period after being opened, its stability during the storage period should be confirmed.

(3) Apply the test substance properly, in a formulation and according to a method of usage (in terms of time, number of applications, quantity, etc.) that is relevant to the application for registration, and using the tools customarily employed.

(4) Do not apply the test substance during rainy weather, or when rainfall is expected shortly after application. However, when rain will not affect results, due to use of a greenhouse, etc., this limitation is unnecessary.

4. Collecting samples (soil)

(1) Method of collection

(i) Gather samples once from 4 different places in each field, and mix well.

(ii) Gather cylindrical samples of at least 200 g, down to a depth of approximately 10 cm below the surface. When the test fields are paddies, collect paddy water as well.

(2) Collection schedule and the number of times collected

Collection samples once each immediately prior to and following addition of the test substance, and at least 4 times thereafter.

5. Handling of samples

(1) Transporting samples

(i) Care should be taken in transporting samples so that they do not deteriorate or become contaminated. They should be transported rapidly, at low temperatures, but not frozen.

(ii) Handle samples properly, by affixing identifying labels, etc., in order to prevent confusion among samples during transport.

(2) Handling samples after transportation

Immediately upon receiving samples, verify their authenticity according to their identification labels, etc. Handle them properly, so as to avoid confusion among samples, and use them promptly for analyses.

6. Study period

In general, set the study period as the time until the analytical values of the target substances (when there are multiple target substances, compute the total amount of active ingredient, based on the analytical values) in test soil decline to approximately 10% of the concentration immediately following addition (if the values do not decline to approximately 10%, the time until they decline to less than 1/2 of the concentration immediately following addition).

7. Analysis of samples

(1) Target substances for analysis

The substances to be analyzed are the active ingredients of agricultural chemicals related to the test substance, as well as substances generated in the course of the biological and chemical changes they undergo. However, this does not apply to substances that leave extremely minute residues, and which have been deemed non-harmful due to their extremely low toxicity, etc.

(2) Method of analysis

(i) Adopt a method by which the target substance can be accurately analyzed.

(ii) Express the amount of target substance residue as concentration in dry soil (mg/kg).

(iii) Analyze each sample at least twice, and use the mean value from these analyses as the measured value.

(iv) Confirm the precision of the analytical method by means of the coefficient of variation within the range of concentrations in which detection of the target substances is anticipated.

(v) The sensitivity of the analytical method should be consistent with study objectives, and should be expressed according to the limit of determination, that is, the minimum concentration at which a recovery rate can be obtained that is sufficient for all operations involved in analysis of samples.

(vi) Confirm the recovery rate of the analytical method, within the limit of determination and the range of concentrations in which detection of the target substances is anticipated, using samples that have been collected from untreated plots, and to which a known quantity of the target substance has been added.

(vii) In general, use samples for analysis promptly after they have been collected. If temporary storage of samples is unavoidable, store them under appropriate conditions, conduct storage stability examinations to determine the stability of target substances during the storage period.

(viii) Conduct storage stability examinations of stored samples, using samples that have been collected from untreated plots, and to which a known quantity of the target substance has been added, under the same conditions, for at least the same period, and according to the same methods by which test samples are analyzed.

8. Items to be reported

(1) Institution preparing study results (field test facilities and institution conducting analyses)

(2) Test substance

(3) Study conditions

(4) Target substances for analysis

(5) Method of analysis (summary and details)

(6) Limit of quantification and recovery rate in each analysis

(7) Method of preparing samples, etc.

(8) Results of analysis

Results should include the analytical values at each sample collection time, decay curve, the time until the concentration declines to less than 1/2 of its maximum value. If multiple components are targeted for analysis, compute the total amount of all active ingredients, and report the time until the concentration for this total amount decline to less than 1/2 of its maximum value.

Studies of residue in succeeding crops (3-2-2)

1. Objective

The purpose of these studies is to obtain scientific information regarding the degree to which agricultural chemicals conveyed via soil leave residue in crops.

2. Test crops

(1) When the test substance is for application to paddies, select at least 2 plants, belonging to different families, from among wheat and other small-type grain, soybeans, and root vegetables.

(2) When the test substance is for application to upland fields, select at least 1 type of root vegetable, and otherwise, at least 1 type of crop thought suitable to be a succeeding crop.

3. Test plot (field) selection

There is no objection to conducting the studies with pots, using crops that are relevant to the application for registration, and field soil conducted according to methods of use that are relevant as well, when it is difficult to ensure, prior to cultivation of the test crops, that fields will be available for this purpose. Otherwise, conduct as in studies of residue in crops.

4. Cultivation of test crops

Conduct as in studies of residue in crops.

5. Handling and conduct of the test substance

Conduct as in studies of residue in crops.

6. Collecting samples

Conduct as in studies of residue in crops.

7. Handling samples

Conduct as in studies of residue in crops.

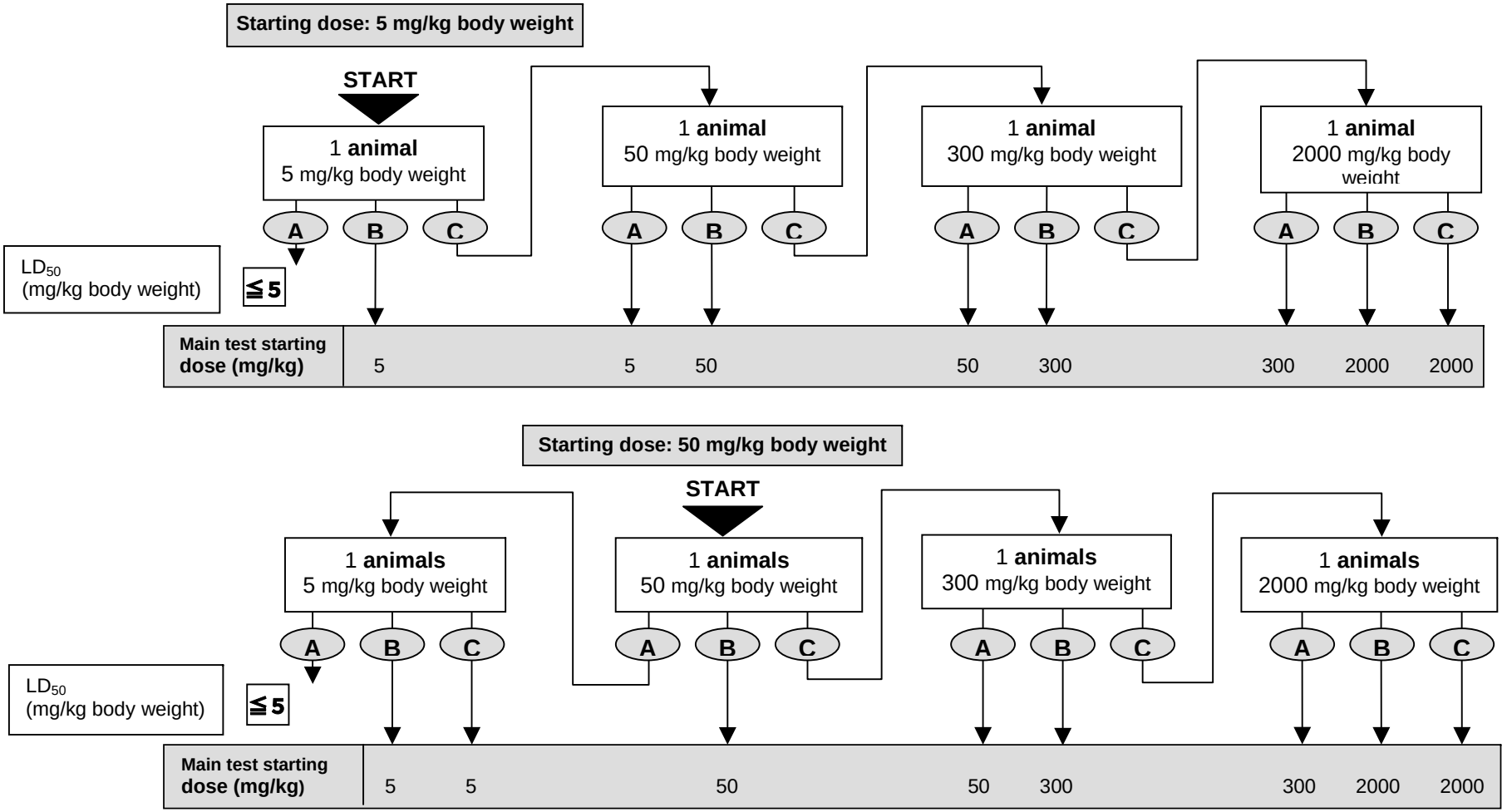
8. Analysis of samples

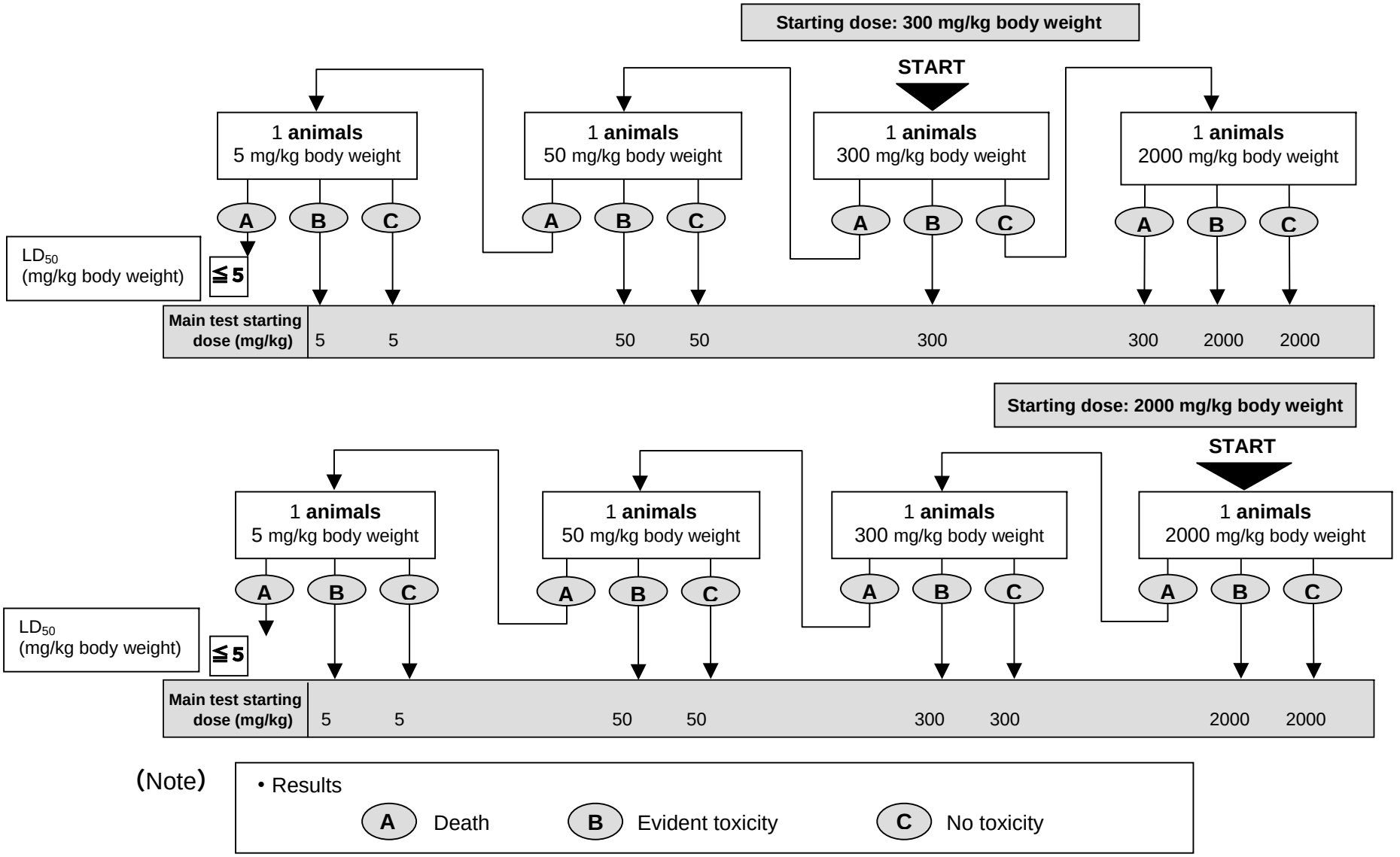
Conduct as in studies of residue in crops.

9. Items to be reported

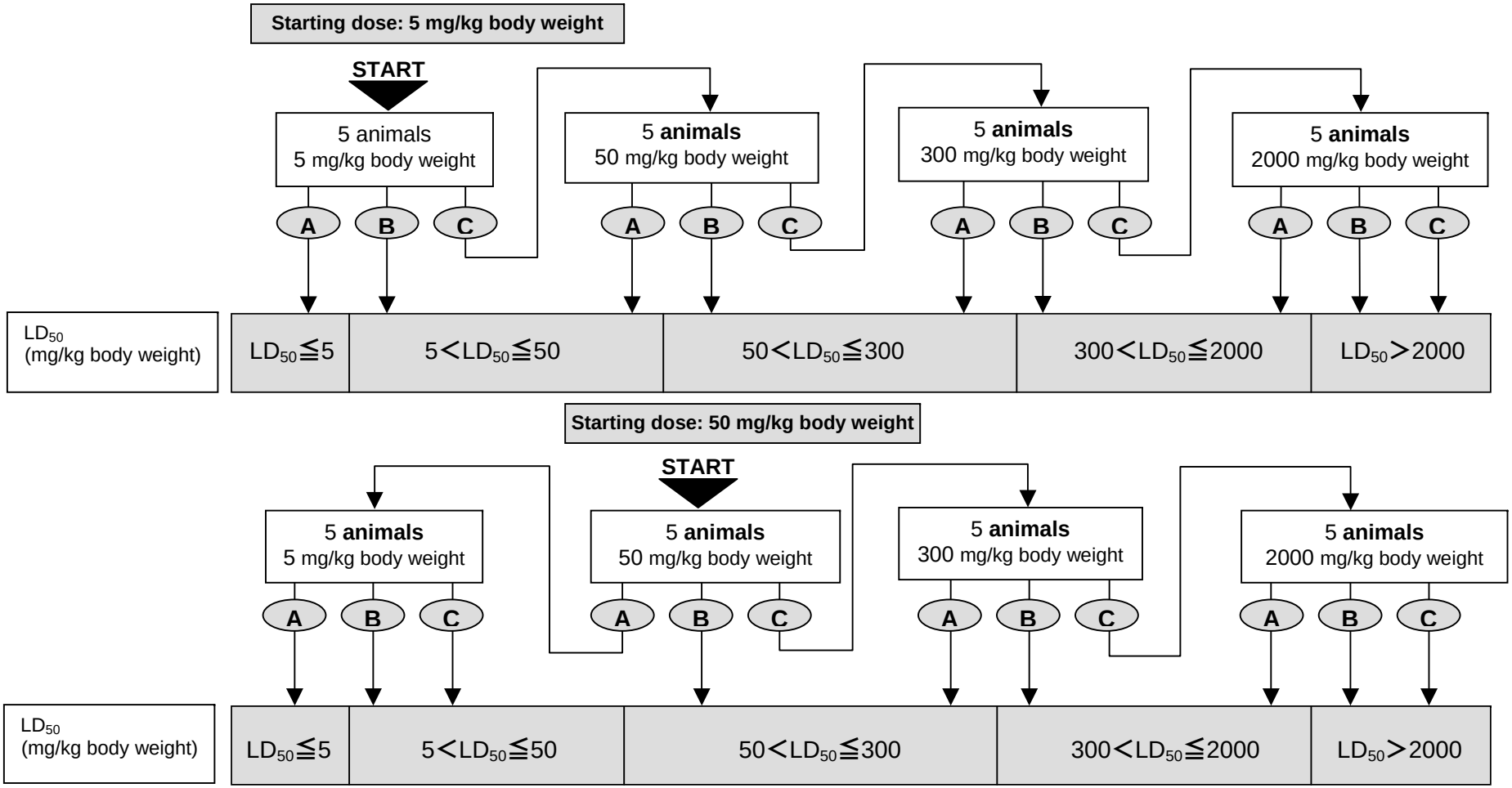
Conduct as in studies of residue in crops.

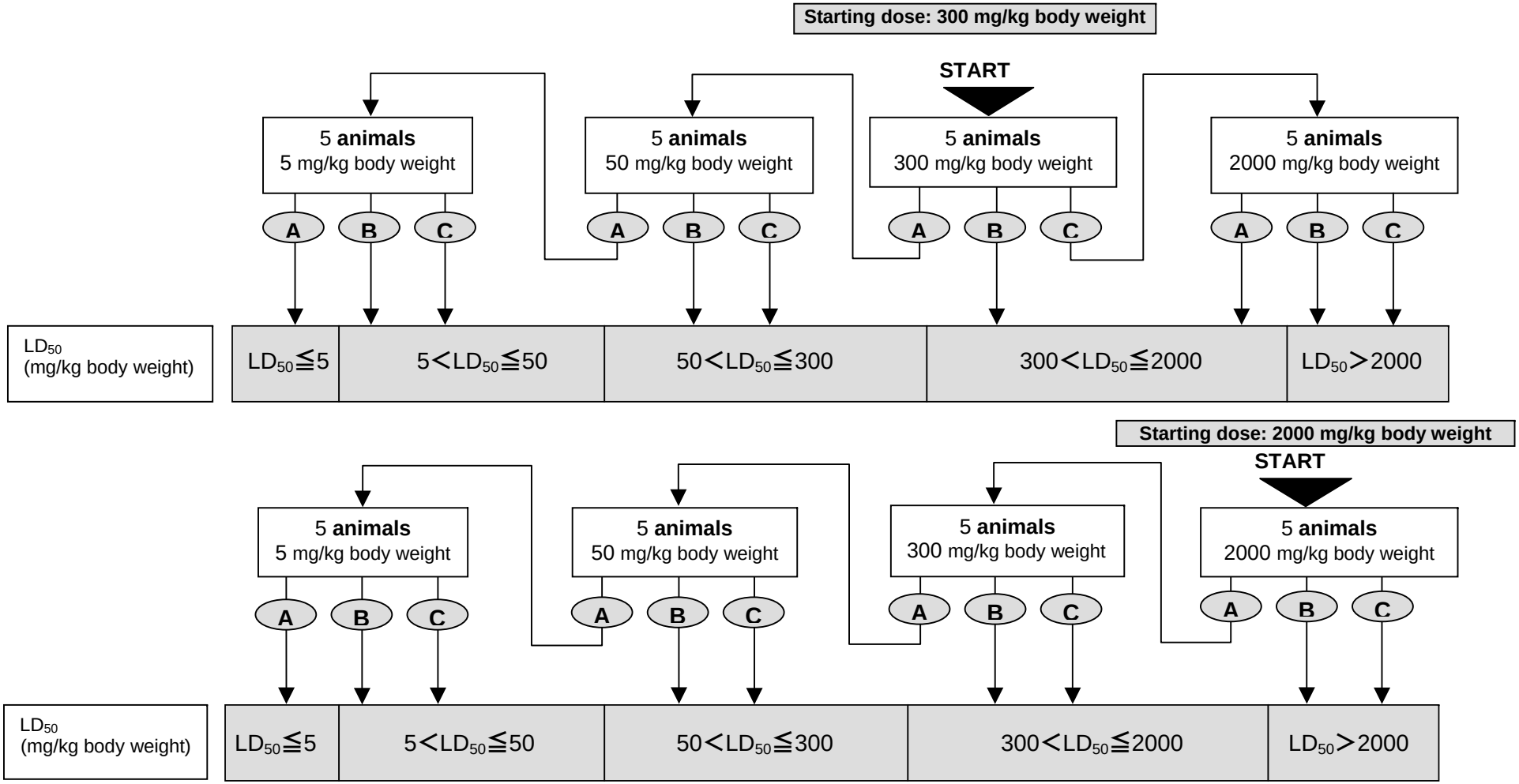
Annex 2－1－1－①：Fixed dose method, sighting study procedure




Starting dose: 300 mg/kg body weight
START
1 animals
5 mg/kg body weight
1 animals
50 mg/kg body weight
1 animals
300 mg/kg body weight
1 animals
2000 mg/kg body weight
A
B
C
LD50
(mg/kg body weight)
≦5
Main test starting dose (mg/kg)
5
5
50
50
300
300
2000
2000
Starting dose: 2000 mg/kg body weight
START
1 animals
5 mg/kg body weight
1 animals
50 mg/kg body weight
1 animals
300 mg/kg body weight
1 animals
2000 mg/kg body weight
A
B
C
LD50
(mg/kg body weight)
≦5
Main test starting dose (mg/kg)
5
5
50
50
300
300
2000
2000
(Note)
• Results
A Death
B Evident toxicity
C No toxicity

Annex 2－1－1－②：Fixed dose method, main study procedure





(Note)

• Results

A ≥2 deaths

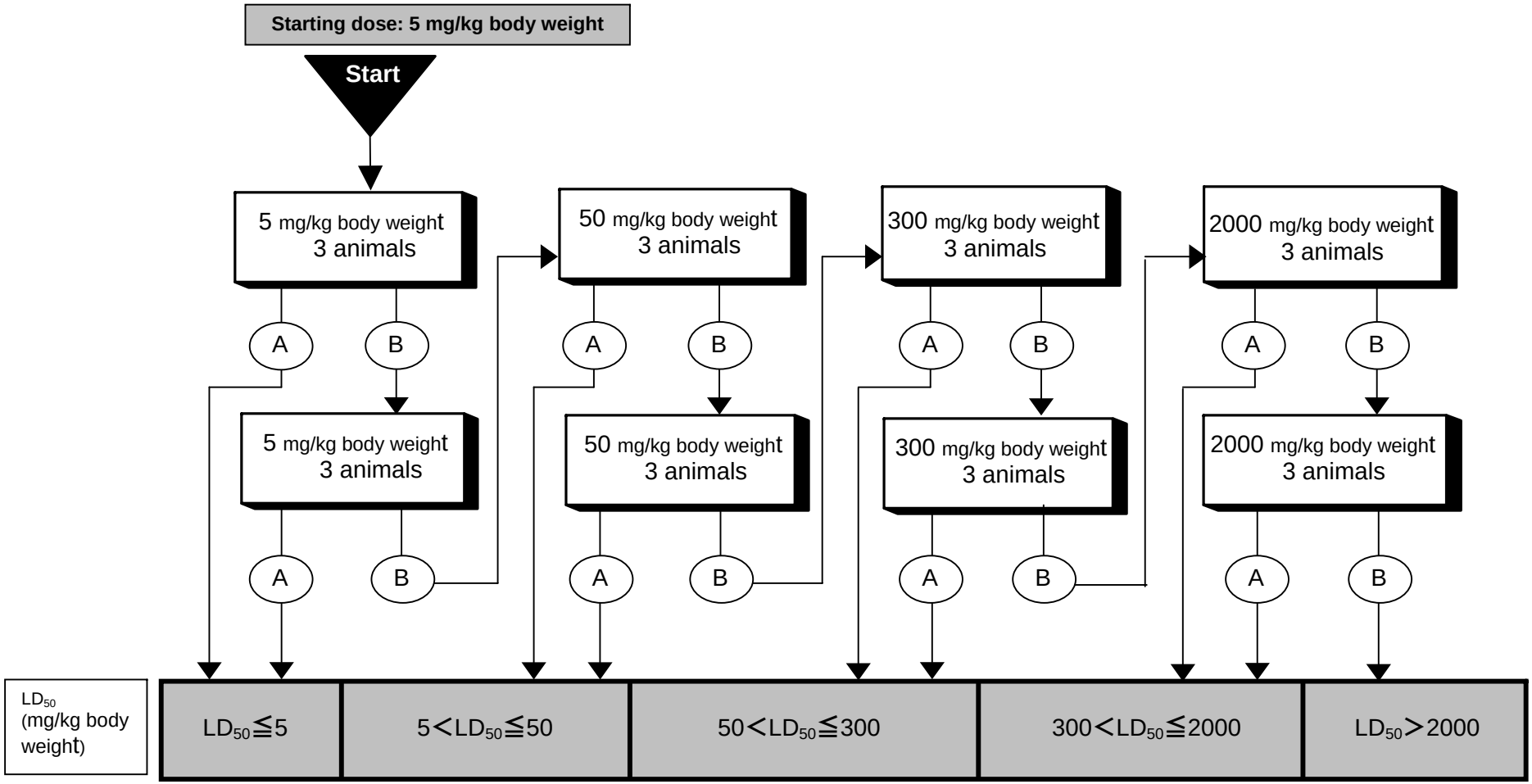
B 1 death,
≥1 animals with evident toxicity, or
1 death and ≥ 1 animals with evident toxicity

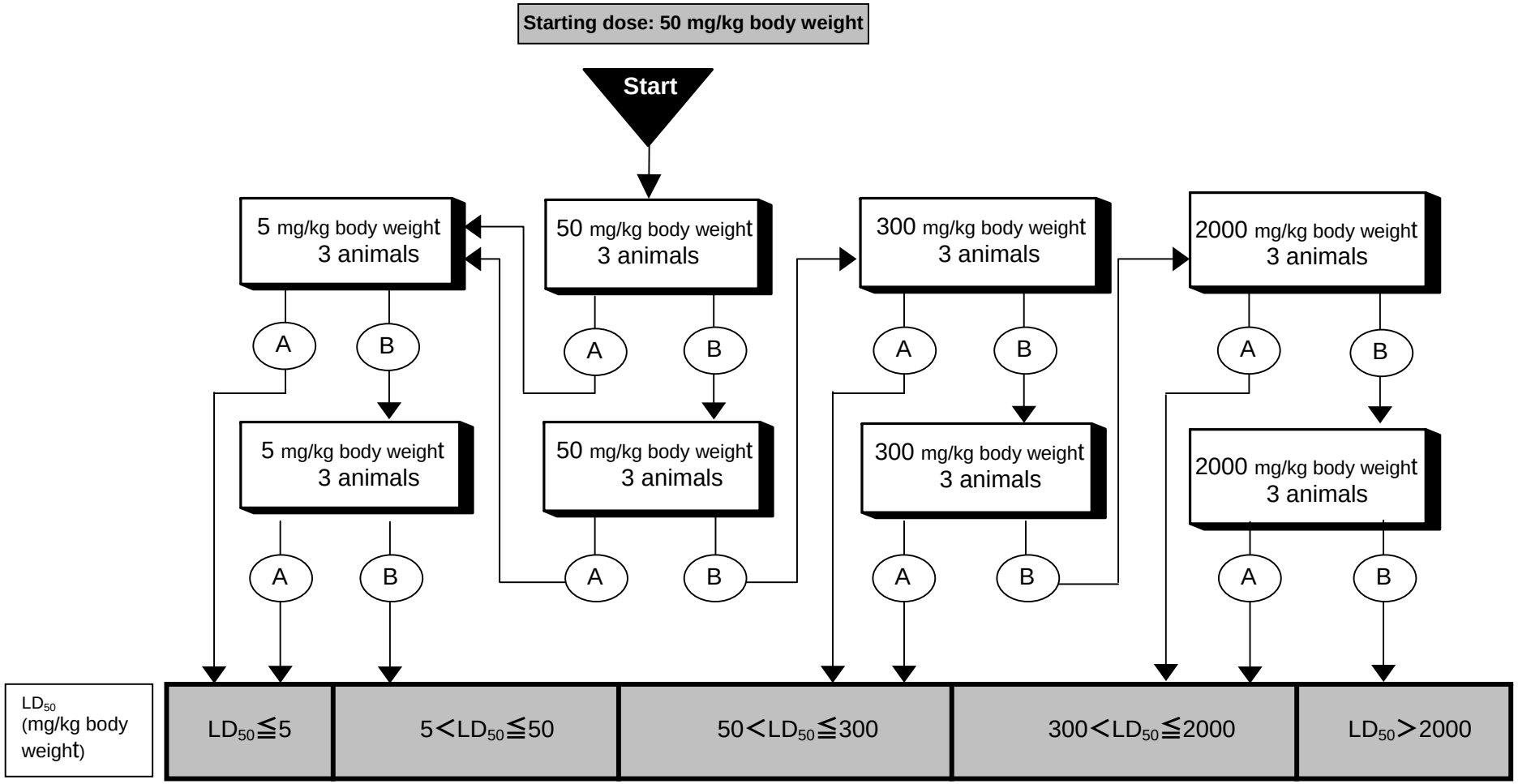
C No toxicity

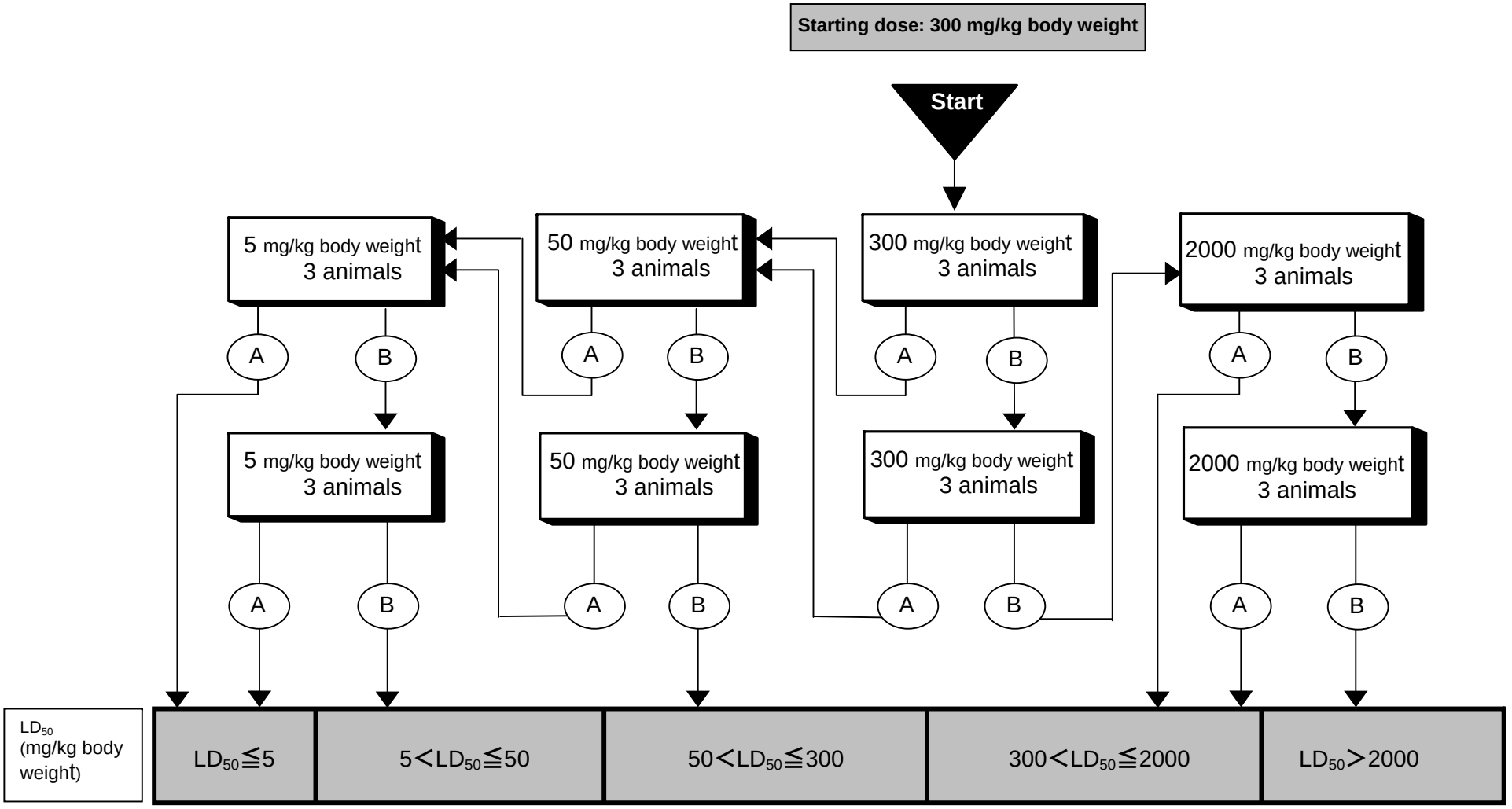
• As regards dosages used in the sighting study, 4 animals were used so that there would be 5 animals, including the 1 additional animal used in the sighting study. In that case, test results were determined for 5 animals, including the 1 additional animal.

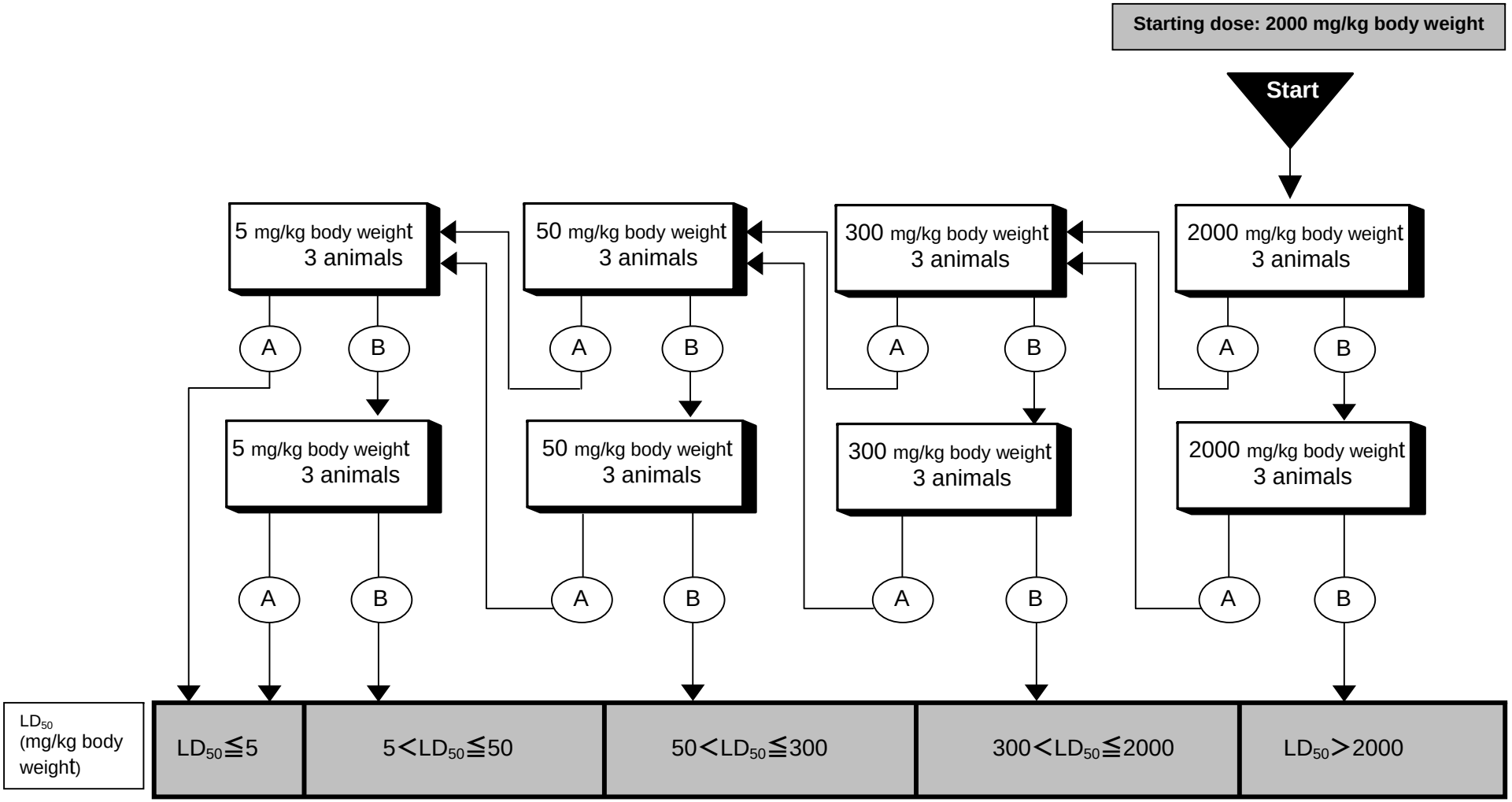
• For dosages at which deaths were noted in the sighting study, the number of deaths in the main study were regarded as ≥ 2, and these dosages were not used in the main study.

Annex 2－1－1－③：Toxic Class Method Procedure




Starting dose: 50 mg/kg body weight
Start
5 mg/kg body weight
3 animals
50 mg/kg body weight
3 animals
300 mg/kg body weight
3 animals
2000 mg/kg body weight
3 animals
A
B
5 mg/kg body weight
3 animals
50 mg/kg body weight
3 animals
300 mg/kg body weight
3 animals
2000 mg/kg body weight
3 animals
LD50 (mg/kg body weight)
LD50≦5
5<LD50≦50
50<LD50≦300
300<LD50≦2000
LD50>2000


Starting dose: 300 mg/kg body weight
Start
5 mg/kg body weight 3 animals
50 mg/kg body weight 3 animals
300 mg/kg body weight 3 animals
2000 mg/kg body weight 3 animals
A
B
5 mg/kg body weight 3 animals
50 mg/kg body weight 3 animals
300 mg/kg body weight 3 animals
2000 mg/kg body weight 3 animals
LD50 (mg/kg body weight)
LD50≦5
5<LD50≦50
50<LD50≦300
300<LD50≦2000
LD50>2000


Starting dose: 2000 mg/kg body weight
Start
5 mg/kg body weight
3 animals
50 mg/kg body weight
3 animals
300 mg/kg body weight
3 animals
2000 mg/kg body weight
3 animals
A
B
5 mg/kg body weight
3 animals
50 mg/kg body weight
3 animals
300 mg/kg body weight
3 animals
2000 mg/kg body weight
3 animals
A
B
LD50 (mg/kg body weight)
LD50≦5
5<LD50≦50
50<LD50≦300
300<LD50≦2000
LD50>2000
• Results
A 2-3 animal deaths
B 0-1 animal deaths
(Animal deaths include animals sacrificed because they were moribund.)