Test methods and Determination Criteria for New Chemical Substances and the Applicability as Monitoring Chemical Substances

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Based on the Act on the Evaluation of Chemical Substances and Regulation of Their Manufacture, etc., the current determination criteria regarding results of the tests required for the determination of new chemical substances as stated in Article 4-1 and 4-2 and determination of applicability to monitoring chemical substances is as outlined below.

With the following criteria as basic rule for the present, determination shall be made with consideration of the expert opinions provided by the relevant councils.

Ⅰ. Test method

(1) Determination as to which items defined in Article 4-1 and Article 4-2 of a new chemical substance falls under, or whether it falls under the category of monitoring chemical substance shall be based on available knowledge pertaining to the ….new chemical substance and existing chemical substances, additionally, it shall be based on the following results of the tests prescribed under Article 1-1 Item 2, Article 1-2 and Article 1-3 of “Ministerial Ordinance Prescribed for the Testing of New Chemical Substances and Items for the Investigation of Hazardous Properties of Priority Assessment Chemical Substances or Monitoring Chemical Substances.”

(i) Ready biodegradability by microorganism (biodegradation test)

(ii) Bioconcentration test of chemical substances in fish and shellfish (bioconcentration test) or octanol-water partition coefficient test (Pow measurement test)

(iii) Repeated dose 28-day oral toxicity study conducting mammals (28-day repeated dose toxicity test), repeated dose 90-day oral toxicity study conducting mammals (90-day repeated dose toxicity test), or combined repeated dose toxicity study with reproduction / development toxicity screening test conducted with mammals.

(iv) Bacterial reverse mutation test, in vitro mammalian chromosome aberration test or mouse lymphoma TK assay (mutagenicity test)

(hereafter, (iii) and (iv) shall be referred to as “screening toxicity test.”)

(v) Freshwater alga and cyanobacterial growth inhibition test, daphnia sp. Acute immobilization test and fish acute toxicity test (ecotoxicity test)

（2） In principle, these tests shall be conducted in accordance with “Test Methods for New Chemical Substances (March 31, 2011, PFSB Notification No. 0331-7, March 29, 2011 Manufacturing Bureau Notification No. 5, Environmental Planning Notification No. 110331009” (Hereafter referred to as, the “Notification”). However, test methods not defined in the Notification (OECD test guidelines, etc.) may be used for determination if the test methods are as equivalent to the above test method (1) and test results are found to be reliable.

Ⅱ. Determination criteria for test results

In principle, the following criteria shall be applied when making a determination based on the results of the tests stipulated in the above Ⅰ. (1).

（1）Biodegradation test

(i)Readily biodegradable

It falls under any of the followings:

・When carried out in three test vessels, the degree of degradation by BOD is 60% or higher in two or more vessels, and the average of the three vessels shall be 60% or higher.

・When carried out in two test vessels, the average degree of degradation by BOD is 60% or higher, and either the degree of degradation in both the vessels is 60% or higher, or the difference in the degree of biodegradation between the highest and the lowest is below 20%.

For determination, it shall be additionally confirmed that no degradation product is formed by direct analysis such as HPLC and GC.

Furthermore, when the results from the tests prescribed by the Notification do not meet the above criteria, determination may be made comprehensively with consideration of test results other than the degree of BOD degradation. Moreover, when the BOD curve indicates that biodegradation continues (such as the rise in BOD) after the test phase, determination may be made based on the test results in accordance with the OECD test guideline 302C.

(ii) Non-biodegradable

・It is not readily biodegradable

（2）Bioconcentration test or Pow measurement test

(i) Highly concentrated

・Bioconcentration factors shall be 5,000L/kg or higher.

(ii) Low concentrated

It falls under any of the followings:

・Bioconcentration factors shall be under 1,000L/kg.

・Common logarithm of n-octanol/water partition coefficient (Pow) shall be under 3.5. However, this shall not apply to surface active substances, mixtures with molecular weight distribution, organic metal compounds, low purity substances (excluding HPLC method) and non-organic compounds.

(iii)When the bioconcentration factor is between 1,000L/kg and 5,000L/kg, the determination may be made comprehensively with consideration of the following test results and knowledge as needed:

・Depuration study

・Bioconcentration factors by specific tissues (edible parts)

・Knowledges pertaining to the concentration dependence

In determining the above, in principle, bioconcentration factors in a steady state shall be used. In the case of (iii), the determination shall be made with consideration of other end points (BCF_{SSL}, BCF_{K}, BCF_{KL}, BCF_{Kg}, BCF_{KgL}). In addition, when BCF values are concentration dependent, tests may be carried out at a lower concentration and the results thereof may be considered for the determination as needed.

Note 1)In case the bioconcentration factor in a steady state cannot be obtained, the determination may be made comprehensively.

Note 2), when it is apparent that the substance is not concentrated in the lipid in the case of (iii), lipid correction shall not be conducted (evaluation shall be made including BCF_{SS}, BC_{FK} and BCF_{Kg}).

Note 3) Regarding test results by specific tissues, evaluation shall be not made based on BCF_L (standardized value with 5% lipid content) but on BCF.

In the dietary exposure method, the test substance shall be determined as not highly concentrated when either the oral bioconcentration factor (BMF_{Kg}) by kinetics with correction applied for growth dilution or the oral bioconcentration factor at the end the uptake period of 10 days or more is less than 0.007. When the BMF_{Kg} is 0.007 or higher, the determination shall be comprehensively made with consideration of the followings other end points (BM_{FK}, BMF_{KL} and BMF_{KgL}), oral bioconcentration factors by specific tissues (edible parts), and test results of reference substances. Regarding determination based on the dietary exposure method, further information shall be gathered and it may be subject to reevaluation as needed.

In addition, when information of the concentration in fish body or properties of accumulation in other living organisms are available, the determination shall be reviewed with consideration to such information, as needed.

(3-1) Screening toxicity tests

(i) Bacterial reverse mutation test

a) Positive

・In any of the test systems, the number of spontaneous revertant conies shows at least 2-fold increase compared to that of the solvent control, and the increase is reproducible or concentration-related.

・If the specific activity value is 1,000 revertant colonies per mg or higher, in principle, the substance is determined as being strongly positive.

・In principle, for positive substances that exhibit poor reproducibility and/or poor concentration-related response, they shall be determined as being slightly positive.

b) Negative

・It does not correspond to being positive.

(ii) In vitro mammalian chromosome aberration test or mouse lymphoma TK assay

a) In vitro mammalian chromosome aberration test

［1］ Positive

・In principle, if a chemical meets the following conditions, the substance is determined as being positive.

(a) In at least one of the test concentrations exhibits a statistically significant increase compared with the concurrent negative control.

(b) The increase is dose-related when evaluated with an appropriate trend test.

(c) Any of the test results are outside the distribution of the historical negative control data

・In principle, the substance is determined as being strongly positive when the D20 value is 10^{-2}mg/ml or lower.

・In principle, positive substances is determined as being slightly positive when they exhibit poor reproducibility and/or poor concentration-related response, or they are determined to be positive at the concentrations only at concentration inducing 50% or higher cell growth suppression.

［2］ Negative

・It does not correspond to being positive.

b) Mouse lymphoma TK assay

［1］ Positive

・In any of the test systems, total mutant frequency (MF) of the negative control exceeds the global evaluation factor (GEF) that is 126×10^{-6},and concentration-related response is observed.

・In any of the test systems, the mutant frequency exhibits a statistically significant increase and the increase is reproducible or concentration-related.

・In principle, the substance is determined as being strongly positive when mutant frequency exceeds four times that of the negative control or when the increase exceeds 400×10^{-6}.

・In principle, positive substances is determined as being slightly positive when they exhibit poor reproducibility and/or poor concentration-related response, or their mutant frequency exceeds GEF but is less than 1.8 times that of the concurrent negative control, or they are determined to be positive only at concentrations inducing 80 % or higher cytotoxicity.

［2］ Negative

- It does not correspond to being positive.

(iii) Repeated dose 28-day oral toxicity study, repeated dose 90-day oral toxicity study, combined repeated dose toxicity study with reproduction / development toxicity screening test conducted with mammals

A hazard assessment value is obtained through dividing a NOEL, etc. of the toxicity tests by the following uncertainty factor products. The basic uncertainty factors used in deriving hazard assessment values according to the determination criteria is as follows:

Interspecies difference：		10
Individual difference		10
Test period	Less than 90 days:	6
	Between 90 days or more and less than12 months:	2
	12 months and more:	1
Adoption of LO(A)EL:		10
Significance of effect(∗1):		1～10

(∗1) An uncertainty factor is added up to 10 for "significance of effect" to any of the following cases revealing significant toxic effects :

- Where there is an onset of toxicologically significant changes such as changes in neurobehavioral toxicity and/or serious histopathological changes, in the assumed grounds for NOEL or other toxicity findings.

・Concerning the effects in of the recovery periodphase, where there are in cases of toxicologically significantvital changes such as neurobehavioral toxicity and/or serious

histopathological changes falling under which correspond to one of the following effects that generate:

a. Histopathological changes that are not reversible during the recovery test period.

b. Delayed toxicity

c. Biochemical changes that are not reversible during the recovery test period.

In addition, regarding effects in the recovery period, considerations shall be given to the degree of reversibility, residual toxicity in the recovery period, the presence or absence of delayed toxicity and whether or not the biochemical changes are attributed to histological changes.

(iv) Reproductive and developmental toxicity study

Concerning reproduction toxicity based on combined repeated dose toxicity study with reproduction/developmental toxicity screening test conducted with mammals, the hazard assessment value is calculated by dividing NOEL, etc. by the following uncertain factor products. The basic uncertainty factors for deriving hazard assessment values are as follows.

Interspecies difference：	10
Individual difference：	10
Adoption of LO(A)EL：	10
Quality of test/Significance of effect(∗2)：	10

(∗2) Regarding the “quality of test/Significance of effect,” an uncertainty factor of 10 shall be added as the “quality of test” when combined repeated dose study with reproduction/developmental toxicity screening test conducted with mammals and one-generation reproduction toxicity study, and an uncertainty factor of 10 shall be added as the “significance of effect” when the endpoint is teratogenicity/offspring mortality that is caused at a lower dose than maternal toxicity. However, when the “quality of test” and the “significant of effect” are both correspond to the aforementioned, the combined value of 10 shall be added.

（3-2）Classification for screening toxicity tests

Based on the determination in accordance with (3-1), hazard classes are assigned each for mutagenicity and general toxicity of a substance. The hazard class with the greatest hazard (i.e., a class with the smallest number) shall be adopted for the substance.

When test results are available for reproduction development toxicity, the hazard class with the greatest hazard shall be adopted in comparison with those assigned to mutagenicity and general toxicity.

(i) Hazard class for mutagenicity

［1］: Hazard class 1 (determination shall not be made solely with the results of the screening toxicity test)

［2］: Hazard class 2 (determined as being strongly positive according to (i) or (ii)）

［3］: Hazard class 3 (determined as being positive (excluding slightly positive) according to both (i) and (ii) (excluding ［2］)

［4］: Hazard class 4 (determined as being positive (excluding slightly positive) according to either (i) and (ii) (excluding ［2］)

［5］：No applicable class (determined as being negative (including slightly positive according to both (i) and (ii))

(ii) Hazard class for general toxicity

［1］: Hazard class 1 (unspecified)

［2］: Hazard class 2 (hazard assessment value 0.005mg/kg/day or lower)

［3］: Hazard class 3 (hazard assessment value higher than 0.005mg/kg/day and lower than or equal to 0.05mg/kg/day)

［4］: Hazard class 4 (hazard assessment value higher than 0.05mg/kg/day and lower than or equal to 0.5mg/kg/day

［5］: No applicable class (hazard assessment value higher than 0.5 mg/kg/day)

(iii) Hazard class for reproduction / development toxicity

［1］: Hazard class 1 (unspecified)

［2］: Hazard class 2 (hazard assessment value 0.005 mg/kg/day or lower)

［3］: Hazard class 3 (hazard assessment value higher than 0.005mg/kg/day and lower than or equal to 0.05mg/kg/day)

［4］: Hazard class 4 (hazard assessment value higher than 0.05mg/kg/day and lower than or equal to 0.5mg/kg/day)

［5］: No applicable class (hazard assessment value higher than 0.5 mg/kg/day)

Notwithstanding the above, when deaths, cancer, long-term disorder, effect on reproductive functions and later generations and other toxicologically serious effects corresponding to the aforementioned are found in chronic toxicity studies, studies for effects on reproductive ability and subsequent generations, teratogenicity studies, mutagenicity studies (micronucleus tests), carcinogenicity studies, studies on metabolic fate, pharmacological studies stipulated in the Notification or other studies with the same purpose as the aforementioned, these tests results shall be taken into consideration in order to determine the hazard class of the said substance as needed.

（4-1）Ecotoxicology test

In principle, PNEC is derived from the results of freshwater alga and cyanobacteria, growth inhibition test, daphnia sp. acute immobilization test and fish acute toxicity test based on the following flow. (in principle, toxicity value derived from the freshwater alga and cyanobacteria growth inhibition test shall be calculated based on growth rates. The same applies hereafter.)

Furthermore, if the results of daphnia magna reproduction test or fish early-life stage toxicity test stipulated in the Notification are available, these shall be used to derive PNEC.

Flow chart to derive PNEC

Results of toxicity tests on aquatic organisms

Are three values of chronic toxicity available? Yes/No Select the lowest NOEC

Are two values of chronic toxicity available? Yes/No Select the lower of two values; the lowest chronic NOEC/5 and acute ACR

Select the lowest values; chronic NOEC/10 and two values of acute/ACR

PNEC=selected value/10

Hazard class assigned according to PNEC value

ACR	Algae		20
	Daphnia	Amines	100
		Other than amines	10
	Fish		100

Note 1) ”Select the lower of two values” ;
selecting the lowest value in comparison with the lowest chronic NOEC/5 of the two values of chronic toxicity and the acute toxicity LC50/ACR in the trophic level lacking in chronic toxicity value.

Note 2) “Select the lowest values”;

selecting the lowest value in comparison with chronic NOEC/10 and the lower of the two value of acute toxicity LC50/ACR in the trophic level lacking in chronic toxicity value

（4-2）Classification for ecotoxicological studies

Based on PNEC derived in accordance with (4-1), the hazard class is assigned.

［1］：Hazard class 1 （PNEC 0.001 mg/L or lower)

［2］：Hazard class 2 （PNEC higher than 0.001 mg/L and lower than or equal to 0.01 mg/L)

［3］：Hazard class 3（PNEC higher than 0.01 mg/L and lower than or equal to 0.1 mg/L)

［4］：Hazard class 4 （PNEC higher than 0.1 mg/L and lower than or equal to 1 mg/L)

［5］：No applicable class (PNEC higher than 1 mg/L)

（5）Determination as to which items in Article 4-1 or 4-2 of new chemical substances falls under

Regarding new chemical substances, determination shall be made as follows based on the conclusion derived according to the criteria stipulated in (1) to (4-2).

(i) when a new chemical substance is determined to be readily biodegradable according to (1), the substance shall be determined as corresponding to Article 4-1 Item 5 of the Act.

(ii) when a new chemical substance is determined to be not readily biodegradable according to (1), not highly bioconcentrated according to (2), to fall under one of [1]-[4] prescribed in (3-2), and to correspond to [5] prescribed in (4-2), the substance shall be determined as corresponding to Article 4-1 Item 2 of the Act.

(iii) when a new chemical substance is determined to be not readily biodegradable according to (1), not highly bioconcentrated according to (2), to correspond to [5] prescribed in (3-2), and to fall under one of [1]-[4] prescribed in (4-2), the substance shall be determined as corresponding to Article 4-1 Item 3 of the Act.

(iv) when a new chemical substance is determined to be not readily biodegradable according to (1), not highly bioconcentrated according to (2), to fall under one of [1]-[4] prescribed in (3-2), and to fall under one of [1]-[4] prescribed in (4-2), the substance shall be determined as corresponding to Article 4-1 Item 4 of the Act.

(v) when a new chemical substance is determined to be not readily biodegradable according to (1), not highly bioconcentrated according to (2), to correspond to [5] prescribed in (3-2), and to correspond to [5] prescribed in (4-2), the substance shall be determined as corresponding to Article 4-1 Item 5 of the Act.

（6）Determination of monitoring chemical substances

when an existing chemical substance is determined as not readily biodegradable according to (1) and highly bioconcentrated according to (2), and it is unclear whether they have long-term toxicity against humans and predatory animals at higher trophic levels, the existing chemical shall be determined as monitoring chemical substance

For substances posing difficulties in evaluation based on the criteria above, determination shall be made on the safe side by referring to the precedent determinations and evaluations on similar substances.

Ⅲ. Determination based on the polymer flow scheme

In principle, the following criteria shall be used for the determination based on the polymer flow scheme.

（1）When a substance fulfill (i) and (ii) as shown below, it shall be determined as being not readily biodegradable and not being highly bioconcentrated

(i) Physiochemical stability test and acid/alkali solubility test

-The criteria for dissolved organic carbon (DOC) change

：In two cycles results, there is no change in DOC before and after the test (the change by 1% or less is considered to be negligible). When there is DOC changes exceeding 1%, physical and chemical stability such as no change in the chemical structure shall be confirmed by other analysis methods.

-The criteria for weight change (It is applicable when the determination by DOC change is irrelevant)

：In two cycles results, there is no change before and after the test (the change by 2% or less is considered to be negligible). When there is weight change exceeding 2%, physical and chemical stability such as no change in the chemical structure shall be confirmed by other analysis methods.

-The criteria for IR spectrum change

：In two cycles results, there is no change before and after the test.

-The criteria for molecular weight change

：In two cycles results, there is no change before and after the test.

(ii) Solubility test in water / organic solvents

One of the following a-c is applicable.

a） There is no change in DOC exceeding in water, and no weight change of test substance in 2% in either of the following two organic solvents. When it is inappropriate to determine from the DOC change in water, there should be no weight change exceeding 2% before and after the test in water.

Water, tetrahydrofuran (THF), dimethylformamide (DMF)

＊ Dimethylsulfoxide (DMSO) or N-methylpyrrolidone (NMP) there is may be used as substitute for DMF.

b） In cases of other than the above a), the content of fractions with molecular weight of less than 1,000 is below 1%.

c） In cases of other than the above a), when the content of fractions with molecular weight of less than 1,000 exceeds 1%, there is no finding suggesting a highly bioaccumulative properties and the fractions with molecular weight of less than 1,000 is unlikely to be bioaccumulative.

Moreover, when a substance does not fulfill the criteria in the above (i) and (ii), determination shall be made based on the results of the readily biodegradability test, bioconcentration test, screening toxicity tests and ecotoxicology tests.

（2） If a substance fulfills the criteria in Ⅲ. (1) (i) and (ii) in addition to (2) a) and c) , it shall be determined as corresponding to Article 4-1 Item 5 of the Act.

a） It does not contain heavy metals and does not indicate any possibility of long-term toxicity to humans based on the knowledge derived from the correlation between the chemical structure and long-term toxicity.

b） In cases of other than a), determination shall be made based on results from the screening toxicity tests.

c） One of the following shall be applicable.

(i) It does not contain heavy metals and is not soluble in water, acid and alkali. It also corresponds to one of the followings.

・Self-dispersibility* in water is not confirmed

・When self-dispersibility in water is confirmed, the main structure does not render cationic properties

* Substances having dispersibility properties in the absence of a dispersing agent.

(ii) It does not contain heavy metals. In addition, its main structure does not render cationic properties, and it does not indicate any possibility to pose a risk of interfering with the inhabitation and/or growth of flora and fauna based on the knowledge derived from the correlation between the chemical

structure and toxicity against flora and fauna when a substance does not containing, it is soluble in water, acid, and alkali.

d） In cases of other than c), determination shall be made based on the results of the ecotoxicology tests.