administered at the highest dose of the active ingredient (*DOSE*: per day per person). The pharmaceutical product is also assumed to be used in a certain fraction (F_{pen}) of the population in the watershed and then emitted into the household effuent to enter into the public water area. There may be multiple routes (ALO_j) from households to the public water area (direct release, after treatment in a wastewater treatment plant, digestion tank, or waste treatment plant). The total sum of the fractional rate for each route ($\sum_{j=1}^{n} ALO_j$) is equal to 1 (100%).

The persistence rate, the fraction remaining without undergoing degradation (or treatment) for each route (PR_j) may vary depending on the characteristics of the target pharmaceutical product and treatment method applied at each treatment/disposal facility. The value of PRj for each route is determined by considering the effects of the final disposal (e.g, environmental pollution due to leakage from garbage landfill). Taken together, expressing the overall persistence rate (the total sum of the persistence rate for each route) as F_{total}, the total amount of the target pharmaceutical product remaining untreated can be expressed as in the numerator of Eq.1. Assuming the total water volume in the public water area as $W_{surface\ water}$, the concentration of the target pharmaceutical product in the public water area ($PEC_{surface\ water}$) is calculated by using Eq.1:

$$PEC_{surface\ water} = \frac{DOSE \times (Population \times Fpen) \times F_{total} \times 10^3}{W_{surface\ water}} \quad [\mu g/L] \qquad \text{--- Eq.1}$$

where $F_{total} = \sum_{j=1}^{n} (ALO_j \times PR_j) \qquad \sum_{j=1}^{n} (ALO_j) = 1$

Item	Symbol	Remarks
Maximum dose	DOSE (mg /person·day)	Maximum amount of the active ingredient administered per person per day (maximum recommended dose) as provided by the applicant
Watershed population	Population (person)	Population in the watershed (entire population of Japan in this model)
Penetration ratio	Fpen	Fraction of the population using the target pharmaceutical product to the total population
Total persistent rate	F_{total}	Overall probability that the target pharmaceutical product remains undegraded (untreated) in various emission routes
Allocation rate	ALO_j	Fraction of the target pharmaceutical product emitted into wasewater treatment plants, digestion tanks, waste disposal sites, direct release, etc. Allocation to Treatment System
Persistent rate	PR_j	The fraction of the target pharmaceutical product remaining undegraded (not mineralized) at each treatment site. Persistence Rate in the Treatment System

Annual watershed water	$W_{surface\ water}$ (m^3/year)	Annual water volume in the watershed
Annual watershed waste water	W (m^3/year)	Annual volume of wastewater from domestic use by the total population in the watershed. Waste Water from Domestic Use
Dilution factor	D	Ratio of water flow to waste water from domestic use. Dilution Rate

To literally apply Eq.1 to individual target pharmaceuticals, the value of each parameter should be determined by considering the situation specific to each target. However, this approach is not suitable for initial or preliminary assessment, because many unknown factors remain and clarification of such uncertainties to determine these parameters requires may become too expensive. Therefore, Eq.1 should be further simplified to facilitate its practical application. For simplicity, the total volume of the target pharmaceutical product is assumed to undergo wastewater treatment. This simplification may be justified considering the following:

Unused pharmaceuticals discarded from households in Japan are mostly collected as solid waste to be incinerated in waste incineration plants (78% of general wastes were treated in this way in 2002). If incinerated under appropriate conditions, the environmental impacts of pharmaceuticals as wastes may be negligible. Furthermore, in 2002, 34% of sewage sludge was landfilled for final stabilization, while only about 4% was landfilled as dehydration sludge or dried sludge.[1)] Since the majority of sewage sludge landfilled is incinerated ash and pharmaceuticals are likely to be mineralized upon incineration, leakage of pharmaceuticals from sewage sludge landfilled after incineration for final disposal may be of minor concern.

In 2002, the penetration rate of water closets in the areas where public sewage systems were available was approximately 60%, while that in areas where public sewage systems were not yet available, but digestion tanks were used instead, was approximately 26%. As a consequence, it would be reasonable to consider that approximately 86% of household effuents in Japan undergo water treatment. Although the efficiency of wastewater treatment by a digestion tank is likely to be slightly lower as compared with that in a wastewater treatment plant, both treatment methods are essentially similar, and it is therefore reasonable to assume that the treatment efficiency of these two methods are almost comparable.

Assuming that 1) the total volume of the target pharmaceutical product enters the sewage system, 2) emission from the sewage system is the sole route of entry into the environment, and that 3) the water volume in the public water area, $W_{surface\ water}$, is expressed by using the water volume in the sewage system (W) and the volume ratio of sewage water to

natural water (D), Eq.1 is transformed to Eq.2. The assumption that the total volume of the target pharmaceutical product undergoes wastewater treatment influences the final decision as follows: ignoring direct release, leakage from the landfill, and difference in efficency between a wastewater treatment plant and a digestion tank on the less safe side; ignoring incineration on the safe side.

$$PEC_{surface\ water} = \frac{DOSE \times (Population \times Fpen) \times (PRwt) \times 10^3}{W \times D} \quad [\mu g/L] \qquad \text{--- Eq.2}$$

The persistence rate at a wastewater treatment plant (PRwt) can be estimated from the biodegradation test results. The persistence rate estimated by a biodegradation test is denoted as Fp (≅PRwt). Assuming that the assessment area includes the whole of Japan, the watershed population is equal to the total population in Japan, 128 million (=Population). Since the annual volume of wastewater from domestic use (W) is 14.2 billion m^3/year = 314 L/person·day[2], the volume of wastewater from domestic use per person per day (Wperson) can be calculated to be approximately 300 L/person·day. Then Eq.2, is further transformed to Eq.3.

$$PEC_{surface\ water} = \frac{DOSE \times Fpen \times F_p \times 10^3}{Wperson \times D} = DOSE \times F_p \times 0.0033 \quad [\mu g/L] \qquad \text{--- Eq.3}$$

In Eq.3, the national average value is assumed for each parameter used for estimating PEC. However, considering the existence of regional differences, such as concentration of the population into urban areas, whether such parameter values are sufficiently on the safe side may be controversial. Estimation of PEC taking the situation specific to a particular assessment area into account should be considered at subsequent assessment steps involving more refined or elaborate methods. Use of the general or representative parameter values should be appropriate for PEC estimation at the screening level. It should also be noted that Eq.3 does not take metabolites into account. PEC estimation involving metabolites is discussed later.

3-2. Estimation of LEVEL 1 Predicted Environmental Concentration ($PEC_{surface\ water\ LEVEL1}$)

To estimate the environmental concentration for surface water, the following assumptions are made in regard to the source of emission and route of entry into the environment:

- The maximum recommended dose is prescribed uniformly to each person taking the target pharmaceutical product.
- There is no variation in the amount of the target pharmaceutical product actually taken by

each person (i.e., no regional difference, no seasonal variation).

- The annual total volume of wastewater is equal to the annual volume of water for domestic use taken from published statistical data.
- Neither the unchanged nor active drug is metabolized in vivo and at wastewater treatment plants.
- Final effluents from wastewater treatment plants are diluted in the environment according to the value of the dilution factor (D) to generate river surface water.

Under the assumption that "not metabolized at wastewater treatment plants (i.e., resistant to wastewater treatment involving biodegradation)", Fp=1 is assumed in Eq.3. Then Eq.3 is transformed to Eq.4, whichi is used to estimate $PEC_{surface\ water\ LEVEL1}$.

$$PEC_{surface\ waterLEVEL1} = \frac{DOSE \times Fpen \times 10^3}{Wperson \times D} = DOSE \times 0.0033 \quad [\mu g/L] \qquad \text{--- Eq.4}$$

Item	Symbol	Unit	Remarks
Maximum dose	Dose	mg/person·day	Maximum amount of the active ingredient administered per person per day (maximum recommended dose) as prodived by the applicant
Penetration ratio	Fpen	—	Default: 0.01
Volume of wasetwater from domestic use	Wperson	L/person·day	Default: 300 L/person·day
Dilution factor	D		Default: D=10

Eq.4 is a very simplified form of Eq.1 and the resulting $PEC_{surface\ water\ LEVEL1}$ solely depends on the maximum dose, reflecting a situation unique to pharmaceuticals, for which the maximum dose is known.

To scientifically derive the threshold limit in surface water (an environmental concentration over which the decision to move on to the next step of the assessment is made), multiple toxicity values should be obtained for each toxicity test required to analyze their distribution. Actually, however, only a limited number of toxicity values are available for pharmaceuticals and it is therefore impossible to investigate their distribution. Under such circumstances, a threshold limit of 0.01 μg/L in the EMEA guideline is adopted considering international harmonization. It should be noted that, even among agricultural chemicals that tend to have relatively high ecotoxicity, only a few are likely to have a PNEC below 0.01 μg/L.[3] Adopting a threshold limit of 0.01 μg/L and using Eq.4, it is concluded that pharmaceuticals with a maximum dose of below 3.0 (mg/person·day) may be exempted from environmental risk assessment.

On the other hand, the relationship between the maximum dose of the target

pharmaceutical product and the annual production limit (A; kg/year) is expressed by the following equation, assuming that the total population in Japan Pop=128 million:

$$A(Kg/year) = \frac{DOSE(mg/person \cdot day) \times Pop(person) \times Fpen \times 365(day)}{10^6}$$

$$= \frac{DOSE \times 128 \times 365 \times 10^6}{10^6 \times 10^2} = DOSE \times 467$$

Using this equation, the annual production limit for a pharmaceutical product for which the maximum dose is 1 mg/person·day is calculated to be approximately 467 kg. Similarly, the annual production limit is 1.4 tons for a maximum dose of 3 mg/person·day and 47 kg for 0.1 mg/person·day, respectively. This relationship demonstrates that consideration of environmental impacts imposes restriction on production of pharmaceuticals in a manner depending on their maximum dose: production of pharmaceuticals with higher potency (effective at smaller doses) tends to be restricted more severely. The annual production limit for a maximum dose equal to 0.01 μg/L (the threshold limit in the EMEA guideline) is calculated to be 1.4 tons, which is far lower than the expected annual production level (over 10 tons/year) inevitably subjected to environmental impact assessment under The Chemical Substances Control Law in Japan. In other words, the EMEA guideline in EU imposes more severe restriction on the production of pharmaceuticals than The Chemical Substances Control Law in Japan, and thereby assures higher environmental safety. It should be noted that application of Eq.4 to a particular pharmaceutical product requires satisfaction of certain conditions. When an Fpen value greater than the default level for Eq.4 (0.01) is expected, this expected value should be used in place of the default.

3-3. Estimation of LEVEL 2 Predicted Environmental Concentration (PEC_{LEVEL2})

Estimation of a more refined value for predicted environmental concentraion ($PEC_{surface\ water\ LEVEL2}$) is to use a more refined value for each of the parameters used in Eq,1. A number of different calculation formulae for PEC to be used for mutually different purposes are presented below.

(1) Estimation of PEC for a particular environmental compartment

When migration to the bottom sediment is suggested (K_{OC}>10,000 L/kg), it is necessary to predict a concentration in the bottom sediment. Furthermore, there may be cases in which the potential for pollution of another environmental compartment (terrestrial or atmospheric) has to be considered in the assessment steps following the one for screening purpose (such cases are beyond the scope of the present report and will not be discussed further).

Consideration of a potential for persistence of pharmaceuticals in a particular area or environmental compartment under particular conditions requires model development and computer simulation. However, no such model has been established to date in Japan. Accordingly, construction of models for each different analytical purpose is urgently needed and examination of their applicability should be a subject of a future study. As for estimation of the PEC in groundwater, an equation proposed in the EMEA guideline is used tentatively. More specifically, Eq.6 derived by simplification of Eq.5 is used.

$$PEC_{groundwater} = 0.25 \times PEC_{surface\ water} \quad \text{--- Eq.6}$$

(2) Incorporation of regional characteristics

Environmental risk management within a smaller land area rather than the entire country is beyond the scope of the present report. However, incorporation of regional characteristics in PEC estimation using Eq.1 is possible by changing the values of individual parameters in Eq.1 (population, penetration rate, allocation rate to each treatment system, annual watershed waste water, dilution factor) to reflect the situation specific to an area.

3-4. Estimation of LEVEL 3 Predicted Environmental Concentration (PEC_{LEVEL3})

3-5. Consideration of Metabolites

(1) Meaning of estimate equation

Pharmaceuticals are metabolized in vivo to generate metabolites. The fractions of metabolites etc., vary from product to product and the values can be determined by studies in human subjects. The (overall) secretion rate is calculated by summing the contents in excretory products, such as urine and feces. Assuming the excretion rate for substance i as f_i, f_1-f_m (m, number of metabolites, including the unchanged drug substance) represents the excretion rate for each metabolite etc.($\sum f_i \leq 1$). For external preparations, entry into sewage due to bathing should also be assumed.

Excreted metabolites etc., are further degraded or metabolized at wastewater treatment plants, which further change the values of the fractions for each substance. In a degradation test using activated sludge (OECD 301), a single unchanged drug substance or metabolite is examined at one time, in principle. When substance j is converted to substance i, the fraction of substance i to the initial level of substance j (conversion rate) is designated $asr_{j \to i}$. If i=j, the parameter represents the fraction of substance i remaining unchanged.

Then, assuming that the number of substances originally present in the influent is m, and the number of substances generated is n, and denoting the fraction of substance i in the

influent as f_i, $F_{pi} = \sum_{j=1}^{m} (f_j \times r_{j \to i})$ represents the persistence rate for substance i in the effluent, where $F_p = \sum_{i=1}^{n} F_{pi}$ .F_{pi}represents the overall persistence rate for substance i. This is the meaning of parameter Fp in Eq.3.

(2) Scope of application

For pharmaceuticals, the fractions of each metabolite generated in the human body (f_i) can be assumed to be known. Therefore, testing systems for determination of F_i involving wastewater treatment following *in vivo* metabolism are discussed below.

In general, a biodegradation tests examine a single substance at one time. Consequently, if ten different metabolites etc. are generated in the human body, the biodegradation test has to be performed ten times. Such cumbersome testing can be simplified as follows:

1) Examine only the unchanged drug substance (and the activated drug in the case of pro-drugs).
2) Examine a mixture of substances to be tested (a sample mixture needs to be prepared by synthesis).
3) Examine substances expected to have significant ecotoxicity first. For a group of substances with similar properties, choose one or two to be examined as representative of the group.

At the end of the biodegradation test, both drug metabolites generated in the human body and their degradation products are likely to coexist in the reaction mixture, which may increase the number of substances to be tested for ecotoxicity in environmental organisms (data essential for environmental risk assessment), thereby complicating the subsequent assessment process. Actually, however, the most important targets of environmental risk assessment are substances present in the final effluent from a wastewater treatment plant. Also, it may well be assumed that microbial community in nature has a degradation potential more versatile than that of functioning in the human body. Taken together, it appears most reasonable to "Examine only the unchanged drug substance and the activated drug" in biodegradation test.

It is noteworthy that ecotoxicity tests may be required only for substances with a persistence rate over a certain limit. The threshold limits for persistence rate adopted in the current version of The Chemical Substances Control Law in Japan and the EMEA guideline in EU are 1% and 10%, respectively. Adopting 10% as the threshold limit, no restriction is imposed on the production of a pharmaceutical product if the persistence rate is below 10%

for the unchanged drug substance and all of its metabolites.

Usually, $\sum F_{pi}=1$. When a reliable value for a fraction undergoing complete degradation (mineralization) is available, the contribution of the mineralized fraction can be eliminated from this equation (making the value of this total sum below 1). However, if the mass-balance in the test system is not appropriate, special care, such as use of the proportional distribution, should be taken.

In simplifying Eq.1, "the worst case" should be assumed in principle, and exclusive application of favorable assumptions should be avoided.

As demonstrated above, estimation of PEC considering drug metabolism and other factors is too complicated to use for routine screening. As a consequence, this approach should be used exclusively at a particular stage of the assessment where more refined estimation of PEC is needed. PEC for the unchanged drug substance and each individual metabolite in surface water ($PEC_{isurface\ waterLEVEL3}$) is calculated by using Eq.3.

$$PEC_{i\,surface\,waterLEVEL3} = \frac{DOSE \times Fpen \times F_{p_i} \times 10^3}{Wperson \times D} = DOSE \times F_{pi} \times 0.0033\,[\mu g/L] \quad \text{--- Eq.5}$$

Item	Symbol	Unit	Remarks
Persistence rate	F_{pi}	—	Persistence rate of metabolite i in the final effluent from wastewater treatment facilities to the dose of the pharmaceutical product (assuming that pharmaceuticals administered to human are excreted into the swage system, undergo wastewater treatment and are released into the final efluent)

Environmental impact assessment using the PEC value ($PEC_{isurface\ water\ LEVEL3}$) calculated for each metabolite, etc., involves comprehensive judgment based on the $\sum(PEC_{isurface\ water\ LEVEL3}/PNEC_i)$, the total sum of the PEC/PNEC calculated for each metabolite, etc. This approach is based on the following assumptions:

1) An ecotoxicity value (PNECi) is calculated for all metabolites etc. to be considered in the assessment.

2) The additivity rule applies to the PEC/PNEC.

When the target metabolite is generated also from another pharmaceutical product or already registered as another pharmaceutical product, the concentrations of the metabolites may tend to be relatively high as compared with those of the unchanged drug substance. However, it is actually difficult to consider generation from other substance in addition to degradation in the management of a single substance .

3-6. Summary

PEC is estimated as follows:

1) $PEC_{surface\ water\ LEVEL1}$ is PEC in river surface water estimated by assuming that the total volume of the target pharmaceutical product administered is directly emitted into the environment, using the following equation:

$$PEC_{surface\ water\ LEVEL1} = \frac{DOSE \times Fpen \times 10^3}{Wperson \times D} = DOSE \times 0.0033\ [\mu g/L]$$

2) Development of methods for estimating the PEC considering more specific situations is urgently needed (e.g., model construction, computer simulation, etc.).

3) $PEC_{groundwater}$ is tentatively estimated by the following equation:

$$PEC_{groundwater} = 0.25 \times PEC_{surface\ water}$$

4) LEVEL 3 PEC with metabolites included in the emission scenario is calculated using the following equation:

$$PEC_{i\ surface\ water LEVEL3} = \frac{DOSE \times Fpen \times F_{pi} \times 10^3}{Wperson \times D} = DOSE \times F_i \times 0.0033\ [\mu g/L]$$

Section 4. Estimation of the No-Effect Concentration

4-1. Ecosystem Models

4-1-1. The Concept of Ecosystem

An ecosystem is a material system consisting of biological communities and the inorganic environment, which is defined in "Convention on Biological Diversity" as "a dynamic complex of plant, animal and microorganism communities and their non-living environment interacting as a functional unit"(Article 2), Consequently, ecosystems in the aquatic environment of a river, a lake and sea are mutually different. Furthermore, ecosystems may change with the area, season and time. In brief, an ecosystem is dynamic in nature, both as an entity and as a concept (See Annex 1 for description of the concept of ecosystme).

4-1-2. Ecosystem Models to be Adopted

In view ofecosystem integrity, environmental risk assessment is required to deal with not only risks at an individual level (effects on cells and tissues within an individual and death as a consequence), but also to deal with the effects observed at higher hierarchal levels, such as population, community and ecosystem. Ecotoxicity data used for the assessment are collected by ecotoxicity tests conducted in test models constructed, by extracting some part(s) of the structure and function of an ecosystem and reconstructing them into reproducible forms. Test models each simulating different aspects of an ecosystem generally have the properties summarized in Table 4-1-2.

Table 4-1-2. Test models Used for Ecotoxicity Testing

1. Natural ecosystem	A part of the natural environment is used for testing. Optimal for environmental risk assessment. Assessment methodologies are not yet established. Requires cost and labor for testing. Difficult to obtain appropriate controls. Control of test conditions are generally impossible (see examples of dead water and running water systems below). Dead water systems: lakes, ponds (including artifitial ponds), mesocosms Running water systems: rivers, artifitial rivers (circulatory, one-way), channel
2. Ecosystem model	Generally involves multiple test species (producers, consumers, and degraders). Setting of testing conditions specific to the objective of the test (e.g., bioconcentration via the food chain) is possible. Both large-scale models such as a particular part of a natural lake isolated using plastic sheets (macrocosm or mesocosm) and small scale models such as 300 mL flasks containing a limited number of test species (microcosm) are available. Requires considerable expertise for assessing test results. Cost and labor for testing vary greatly, depending on factors such as test scale. Involves extrapolation of test results to estimate impacts on the natural environment. Test objectives and assessment methodologies depends on the test model used.

3. Species model	Involves a single test species in principle. Generally divided into two groups: long-term (chronic) tests to observe impacts on the test species throughout its lifetime and life history; and short-term (acute) tests to examine impacts on the test species for a part of its life history. Not directly related to an actual ecosystem. Difficult to extrapolate test results to natural ecosystem. Construction of a test system involving multiple test species and development of criteria for comprehensive judgment are essential. Testing methods are relatively simple and inexpensive and data collection is easy. Frequently conducted at the initial step of a stepwise assessment procedure.

So far as the currently available findings are concerned, it is difficult to obtain data on the impacts for a community or higher level of hierarchy (e.g., a risk of decrease in diversity in populations conceiving a risk of total destruction of the entire ecosystem) as a part of routine testing. The most realistic approach available for such a purpose is to conduct tests to examine the impacts on a population (constituent of a biological community) and then to assess the risk on the ecosystem based on the data obtained (e.g., "A potential exists that the observed decrease in population affects diversity at the community level and then an ecosystem"). Therefore, a combination of multiple tests each involving a single test species should be used.

4-2. Test Species

Test species used for ecotoxicity testing in Japan should meet the following requirements: clear ecological significance (e.g., role in an ecosystem), reasonable price, capable of being tested throughout the year, high sensitivity, easy handling (i.e., size, setting of test conditions, etc.), viability in the domestic environment. Practically, test species and testing methods are selected from internationally agreed methods and test species recommended for each method. In conducting general ecotoxicity assessment, aquatic environments are considered to be the entry point of chemical substances into the natural environment and aquatic organisms are chosen as the test species: aquatic plants (algae etc.) as producers, Crustacea (Daphnia etc.), as primary consumers, and fish as predators. For chemical substances with a considerable potential for partition into the bottom sediment predicted from environmental partitioning data, the testing methods would involve benthic organisms as test species.

Some pharmaceuticals may affect activated sludge, which plays an important role in wastewater treatment and thereby damage the capacity of wastewater treatment plants. Therefore, pharmaceuticals with a potential risk of affecting microbial populations should be tested for their effects on microbial metabolism (respiration inhibition) as well.

4-3. Testing Methods

4-3-1. Selection of the General Testing Methods to be Applied

OECD tests (see Annex 2) are globally adopted testing methods for assessing environmental impacts. As a member of OECD, Japan has been involved in these tests since the development of the test guidelines and utilized them for assessing the environmental impacts of chemical substances other than pharmaceuticals. OECD tests are systematically developed depending on the environmental compartment and trophic level to be assessed: algae (producer), Daphnia (primary consumer), fish or birds (predator), bloodworms (benthic organisms) for aquatic environments; higher plants (producers), earthworms or Enchytraeina (consumers), birds (top predators), microorganisms (degraders) for the terrestrial environment. Insects (honeybees) are used as test species exclusively in the assessment of the impacts of chemical substances that have some special usage, such as agricultural chemicals. Rodents are used as the test species in the assessment of the impacts on human health rather than in those of the impacts on the environment. When birds and rodents are used as the test species, clarification of the relationship between the observed impacts and the *in vivo* concentrations of the target chemical substance in the prey species is a key task of assessment, because the major route of exposure to chemical substances for such predator species is considered to be incorporation of such substances accumulated in the body of the prey species closer to the bottom of the food chain.

In the emission scenario for pharmaceuticals in this report, emission into the terrestrial or atmospheric environment is not assumed as the main route. Therefore, test species such as terrestrial organisms are not used in the assessment of the environmental impacts of pharmaceuticals. Since most test models routinely used for ecotoxicity testing involve a single test species, a basic set consisting of multiple tests is used, considering the ecological position of the test species and the interspecies difference in sensitivity. In the case of pharmaceuticals for which a unique environmental fate is expected, additional tests should be considered.

4-3-2. General Testing Methods to be used in the Stepwise Assessment Procedure

Ecotoxicity tests are divided into two categories, namely, short-term (acute) toxicity tests and long-term (chronic) toxicity tests. In stepwise procedures for environmental impact assessment, these ecotoxicity tests are incorporated in two different ways: 2-step strategies involve short-term tests for screening and long-term tests conducted subsequently only for those target substances that are found to be problematic at the screening step; on the other

hand, the 1-step strategies include only the long-term tests. The current version of The Chemical Substances Control Law in Japan basically adopts a 2-step strategy (acute to chronic), while the EMEA guideline (2006)[1)] in EU follows a 1-step strategy (chronic only). Environmental impact assessment for pharmaceuticals proposed in the present report adopts a 1-step strategy (chronic only), keeping international harmonization in mind, based on the following considerations:[1)] Growth and/or reproduction rather than viability of environmental organisms is important as the endpoint in environmental impact assessment; 2) extrapolation of data obtained from short-term tests to predict the long-term effects is fraught with difficulties; 3) there is a global tendency towards shift towards long-term exposure tests of the mainstream of future environmental impact assessment.

The basic set of long-term (chronic) toxicity tests adopted at present proposal involves OECD TG201 (algae) or TG221 (duckweeds), TG210 (fish) and TG211 (Daphnia). The NOEC obtained from TG201 (algae) alone should not be regarded as reflecting a complete assessment of long-term toxicity. In addition to the basic set, OECD TG206 (bird) and TG218 (bloodworm) are adopted as long-term toxicity tests in top predators and bottom sediment toxicity test, respectively. Impacts on wastewater treatment should be test with GLP. Since development of new testing methods by OECD is still in progress and is expected to continue in the future, a flexible assessment system should be developed to allow immediate incorporation of any new testing method following after it is approvedal.

Table 4-3-2. OECD tests adopted in environmental impact assessment for pharmaceuticals proposed in the present report

No.	Test species	Test period	Endpoints
201	Algae	72 h	NOEC; Growth inhibition
221	Duckweeds	7 d	NOEC; Growth, propagation
211	Daphnia	21 d	NOEC; Number of babys (reproduction)
210	Fish	depends on species	NOEC; Death, hatch, body length and body weight, abnormal behavior/morphology
218	Bloodworm	depends on species	NOEC; Eclosion, death, growth
206	Birds	Parents: 8+(8-10) w Young birds: 14 d	NOEC; Death, body weight, food consumption, pathological observations, egg production, number of abnormal eggs, egg shell thickness, viability of young birds, incubation ratio
209	Activated sludge	0.5-3 h	EC50; Respiration inhibition

4-3-3. Tests for Pharmaceuticals with Unique Actions (Hormones, Antibiotics)

1) Endocrine-disturbing actions

Endocrine-disturbing actions of hormones and chemical substances with hormone-like actions cannot be tested by the testing methods mentioned above, and separate testing methods need to be developed for this purpose. Testing methods for endocrine-disturbing actions are currently being developed by OECD (screening assay in fish to full-lifecycle test in Crustacea), but they have not yet been adopted formally. Thus, assessment of endocrine-disturbing actions should be reconsidered after further methodological advances.

2) Impacts on wastewater treatment plants

Antibiotics/bactericidals pose a potential risk of affecting the functions of wastewater treatment plants, considering the purpose of their use. If the predicted concentration of the target pharmaceutical products in the sewage influent exceeds its effective concentration level, OECD TG 209 (activated sludge) would be required. The concentration in the sewage influent is estimated by assuming D=1 in Eq.4.

4-4. Application of (Q)SAR)

Structure-activity relationships (SAR) is an approach in which the relationships between the biological activities of chemical substances and their physicochemical properties or structure are elucidated, and then these relationships are expressed in the form of a mathematical formula. SAR involving quantitative treatment is designated as quantitative SAR and abbreviated hereafter as (Q)SAR.

4-4-1. Current Status of use of (Q)SAR

Regulatory use of (Q)SAR is frequent in the United States.[1)] The background for this is that submission of ecotoxicity data on application for approval of new chemical substances is not required in the United States, but instead, ecotoxicity prediction by (Q)SAR is a prerequisite; ecotoxicity testing in environmental organisms is required only when (Q)SAR results suggest a potential risk of ecotoxicity. The Environmental Protection Agency (EPA) has developed a QSAR system, called ECOSAR (Ecological Structure Activity Relationships), which is open to the public for use free of charge. The EU has developed the EUSES (European Union System for the Evaluation of Substances) by modifying the USES (Union System for the Evaluation of Substances), a (Q)SAR system originally developed in the Netherlands. These systems were developed for preliminary assessment of chemical substances and are capable of assessment including exposure. However, estimation of

ecotoxicity by (Q)SAR was not yet implemented in these earlier systems. The OECD has published correlation formulae for (Q)SAR based on a 4-category classification system of chemical substances according to their properties. All these (Q)SAR systems mentioned above are intended to deal with common chemical substances and none is dedicated to pharmaceuticals.

In drug development today, (Q)SAR analysis is almost indispensable for reducing the costs of development, discovery of new chemical structure related to biological activity, and avoidance of toxicity. Advances in computer technology stimulated the development of (Q)SAR. A wide variety of technologies, such as introduction of 2-dimensional or 3-dimensional structure parameters and quantum chemical parameters, analyses of 3-dimensional structures of complexes with receptors (proteins), estimation involving neural networks and data mining, are being developed, and major pharmaceutical companies have constructed their own databases for (Q)SAR. However, it should be noted that all of these systems have been developed to facilitate drug development, targeting the biological activities of chemical substances together with their toxicities to humans, but not considering their ecotoxicity. (Q)SAR analysis requires a sufficient volume of data as a prerequisite. Considering that ecotoxicity data of pharmaceuticals currently available are still limited, it would be reasonable to say that a (Q)SAR system for pharmaceuticals remains to be established.

In Europe and the U.S, (Q)SAR has attracted the interest and expectation of the regulatory authority as well as the industry. In 2004, OECD published a guideline on ecotixicity assessment involving (Q)SAR . In this guideline, information listed in Annex 3 is required for regulated use of (Q)SAR. Also, EU has implemented the idea of a "(Q)SAR Tool Box".[2)]

4-4-2. Usage and Accuracy of (Q)SAR

(Q)SAR is currently used to assist testing processes in industrial utilization of chemical substances, such as 1) assistance of priority determination, 2) development of testing plans, and 3) elucidation of toxicity mechanisms. It can also be used, instead of conducting actual tests, to collect experimental data in such stages as 4) classification, 5) estimation of the lacking data used for placement into chemical classes and labeling, and 6) estimation of the lacking data in risk assessment.

Although use of (Q)SAR is permitted to obtain data required in the examination of application for new drugs in the United States, the final decision is made by experts, and therefore, is not a mechanical judgment. It is worthy of note that experimental data are considered to be superior to predicted or estimated data in any assessment system.

EPA has investigated the effectiveness of ECOSAR in collaboration with EC.[3] The results are summarized in Table 4-4-3-1. ECOSAR yielded good estimates of ecotoxicity in fish and Daphnia, but exhibited a greater tendency to fail in estimating the long-term toxicity as compared to short-term toxicity. This may be partly explained by the lack of observed data in algae in the setting of long-term toxicity tests.

Table 4-4-3-1. Accuracy of Estimation by (Q)SAR

Fish	n	%	Daphnia	n	%
	130	100.0		127	100.0
Concordant	107	82.3	Concordant	90	70.9
Non-concordant	23	17.7	Non-concordant	37	29.1
Overestimation	14	10.8	Overestimation	20	15.7
Underestimation	9	6.9	Underestimation	17	13.4

Note: "Concordant" indicates a deviation wihtin ±1 (log).

4-4-3. A View on (Q)SAR in Environmental Impact Assessment for Pharmaceuticals

To date, no effective application of (Q)SAR to pharmaceuticals has been demonstrated. Although (Q)SAR has the potential to serve as an effective tool in drug management in the future, environmental impact assessment for pharmaceuticals proposed in the present report does not adopt estimation of PNEC using data estimated by (Q)SAR.

4-5. Estimation of No-Effect Level (PNEC)

Several different methods are available for estimating the PNEC based on the ecotoxicity test results, depending on the quality of the data and number of ecotoxicity tests yielding data of acceptable quality. These methods are roughly classified into two groups, those involving an extrapolation factor and those involving a statistical approach.

4-5-1. Extrapolation Using Uncertainty Factor

A commonly used method for estimating the PNEC involves application of an estrapolation facter termed "uncertainty factor" (UF) to the results obtained from toxicity tests. The following model is assumed to derive UF:

$$UF_t = \prod_{i=1}^{n} (UF_i)$$

Here, UF_t denotes the cumulative uncertantiy factor; UF_i denotes the uncertanty factor for test conducted in the ith step of the assessment procedure and depends on the level and

type of the test (n, total number of different extrapolation steps involved). In general, the following four extrapolation steps are assumed: long-term toxicity data to environmental impacts (i=1), short-term toxicity data to long-term toxicity data (i=2), single test species to multiple test species (i=3), intra-species difference in sensitivity (difference in sensitivity between two fish species, e.g., rainbow trout and red killifish). Of these four steps, UF_4 [intra-species difference in sensitivity (i=4)] is assumed to be already considered, based on the recognition that species exhibiting an appropriate sensitivity to the target chemical substance have already been selected from other taxonomically related organisms as representative test species for the particular ecotoxicity test. UF_3 [single test species to multiple test species (i=3)] is not considered in the present proposal, because it involves a combination of three different ecotoxicity tests in three different test species, each representing a different function in the ecosystem (producer, consumer and predator). When only short-term ecotoxicty data are available, UF_2 needs to be considered, but the UF_2 value may vary depending on whether or not the acute/chronic toxicity ratio (A/C ratio) for each species is taken into consideration (see Annex 4 for detailed discussion on A/C ratio). For UF_1[long-term toxicity data to environmental impacts (i=1)], a tentative value of 10 is generally used, but for no clear reason.

For example, OECD recommends the following UF values depending on the situation: when acute toxicity data are available for all three test species (algae, Daphnia and fish), each of which plays a different key role in the food chain, use UF_1=10 and UF_2=10, making UF_t=100; when chronic toxicity data are available for all three test species, use UF_t=UF_1=10.

In the present proposal of environmental impact assessment for pharmaceuticals, only UF_1 is considered, and a value of 10 is tentatively used, because three long-term toxicity tests are conducted in combination in the three key test species for screening purposes.

4-5-2. Extrapolation Involving a Statistical Approach

When only a small number of test data is available for a combination of toxicity tests involving multiple test species, the smallest toxicity value obtained is often used, to be on the safe side, for estimation of the PNEC. In contrast, when multipe data sets are available, a 5th percentile value can be calculated by applying the statistical theory to the obtained data and used to estimate the PNEC (see Annes 5 for details). For environmental impact assessment for pharmaceuticals proposed in the present report, statistical extrapolation is not adopted, because there are scarce chances of obtaining multiple data sets from long-term toxicity tests.

4-5-3. Utilization of Existing Data

In some cases, the applicant would like to retrieve existing toxicity data from the literature or database and utilize them for environmental impact assessment of the target pharmaceutical product. In such cases, it is generally difficult to confirm if the reliability of the tests conducted to obtain such data conforms to the level required by GLP standards, and whether the testing methods and test species involved conform to the relevant guidelines. Since criteria for decision at the regulatory level should be clear and definite, only data obtained by authorized GLP organizations should be used, in principle, for environmental impact assessment for pharmaceuticals and further addition of existing data to complement the lack of actual data should not be permitted. However, in the above-mentioned case, it would be appropriate to develop some regulatory procedure to examine the effectiveness of adding existing data and decide whether or not they should be accepted. Also, it would be appropriate to permit the regulatory authority to utilize existing toxicity data as a basis for calling for some special test for a particular pharmaceutical product.

4-6. Handling of Multiple Pharmaceutical Products

4-6-1. Handling of Multiple Pharmaceutical Products

For pharmaceuticals, the mechanisms of their actions in the human body underlying their biological activities are known in principle. Since there are many pharmaceuticals exhibiting identical efficacy with identical mechanisms of action, their overall environmental impacts are assumed and there remains the concern that environmental impact assessment conducted separately for each of these products are not sufficient to secure environmental safety. A possible approach to address such circumstances is to sum up the emission volume of pharmaceuticals into the environment for each group divided based on the mechanisms of action, to multiply the potency or activity of each product by its emission volume, and finally to take to total sum of contribution by each pharmaceutical product for control of the overall impact. The details of such an approach is described in Annex 6, but is left as a subject for future study.

4-6-2. Multiple Impacts Caused by Multiple Pollutants

The aquatic environment is generally polluted by multiple pollutants。 When multiple substance coexist, toxicity may be decreased by interaction in some cases, while it may be increased in an additive or synergistic manner in others. Although multiple impacts caused by multiple substances may be an important factor to be considered in refining environmental

impact assessment, no general rule for handling multiple impacts is available to date. Accordingly, proper handling of multiple impacts should be left as a future challenge.

4-7. Summary

- Predicted no-effect concentration (PNEC) are estimated using the following methods:

1) Test models each involving a single test species are used in principle. A basic set as a combination of multiple ecotoxicity tests is conducted first for screening purposes. Additional tests are considered for pharmaceuticals for which a unique environmental fate is expected.
2) Only impacts on the aquatic environment are considered at present.
3) The basic is a combination of three different long-term (chronic) toxicity tests, on OECD TG201 (algae) or TG221 (duckweeds), TG210 (fish) and TG211 (Daphnia). The NOEC obtained in TG201 (algae) should not be regarded as a complete result of long-term toxicity when assessed alone.
4) Two additional tests are adopted for environmental risk assessment under unique circumstances: OECD TG206 (bird) as long-term toxicity test in top predators; TG218 (bloodworm) as a bottom sediment toxicity test.
5) Of the toxicity values obtained from the basic set and the bottom sediment toxicity test, the lowest value (indicating the highest toxicity) is divided by the uncertainty factor (UF=10) to estimate the PNEC. PNEC estimation from the toxicity value obtained by the additional test OECD TG206 (bird) remains to be established

- Other comments related to PNEC

6) Estimation of PNEC using data estimated by (Q)SAR is not adopted at present.
7) Whether environmental impacts of mulitple pharmaceutical products with identical mechanisms of action should be assessed after integration is left as a subject for future study.
8) Handling of multiple impacts should is left as a future challenge.

Section 5. Risk Assessment for Environmental Impacts Posed by Pharmaceuticals

5-1. Basic principles of Risk Management

In brief, risk management for impacts of chemical substances on ecosystems is to reduce the risk by 1) suppression of generation, 2) restriction of use, 3) restriction of emission or disposal, 4) actions for restoration, and 5) information disclosure and communication. "Suppression of occurrence", directly leading to reduction of exposure to toxic substances, is expected to be the most effective measure. This approach was applied to reduction of harmful products and by-products generated unintentionally (e.g., dioxins) and yielded remarkable results. "Restriction or prohibition of production/use" of a particular chemical substance based on its risk estimated by risk assessment has been implemented in the form of the following Japanese laws: The Law Concerning Examination and Regulation of Manufacture and Handling of Chemical Substances (Chemical Substances Control Law); The Agricultural Chemicals Regulation Law; and Law Concerning the Protection of the Ozone Layer through the Control of Specified Substances and Other Measures. As for "restriction of emission", The Air Pollution Prevention Law and The Water Pollution Prevention Law have been established to restrict emission volumes and concentrations of chemical substances into the atmosphere and aqueous environment, while The Waste Management Law regulates methods of disposal of chemical substances. Law concerning Reporting, etc. of Releases to the Environment of Specific Chemical Substances and Promoting Improvements in Their Management (Pollutant Release and Transfer Register (PRTR) Law) is aimed at the promotion of "information disclosure" on and voluntary improvement of the management of chemical substances. In addition, The Anti-Farm Soil Pollution Law deals with "restoration" of contaminated soil. More details of the Japanese laws mentioned above are provided in Annex 8.

Risk management related to environmental impacts of pharmaceuticals is a part of the comprehensive policies for management of chemical substances, and "restriction of use" is the most basic approach to be adopted. Besides, "suppression of emission" should also be stressed upon, because disposal of drugs unused at home needs to be minimized for prevention of the environmental risk posed by them by informing consumers about the proper handling of unused drugs, including return to the pharmacist dispensing them or the drugstore selling them.