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Recent Trends Concerning the Ecological Risk Assessment of Chemicals – Following the Revision of the Chemical Substances Control Law –

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1 Introduction

The decline of biodiversity has become one of the key issues concerning the global environment in recent years. Presently, living species of 10 million to 100 million are said to exist on the earth. An immense number of species contributes to the conservation of a wide variety of gene pools to allow numerous ecosystems to exist in various parts of the earth. The variety of ecosystems is a key to securing stable natural circulation of energy and substances from the earth level to the regional level.

The collapse of biodiversity will lead to the disappearance of genetic resources and deterioration of ecosystem functions, thereby damaging the basis for human activities in the long run. The habitat destruction of species is the biggest factor behind the significant decline in biodiversity in recent years. Environmental pollution caused by artificial chemical compounds is considered a serious problem comparable to the destruction of a tropical rain forest.

The main focus of the safety assessment of chemical compounds has been their effects on human health. This, however, is changing because there has been an ever-increasing focus on biodiversity conservation on a global basis and that calls for the assessment of the effects of chemical compounds on other wildlife. Based on this idea, ecological risk assessment for chemical compounds has been considered worldwide. However, as the chemical industries are important economic infrastructures, ecological risk assessment for chemical compounds must be restricted by the sense of economy.

In practice, administrative bodies standardize assessment methods with a specific focus on domestic trade. In the face of the global free market, however, the world is moving toward the establishment of standardized testing and assessment methods. In this article we will explain the status of the ecological risk assessment for chemical compounds both in Japan and abroad, and discuss the future direction of the ecological risk assessment.

2 OECD's effort for ecological risk assessment for chemical compounds

OECD takes the lead in standardization for the ecological risk assessment for chemical compounds. The OECD test guidelines for toxicity of chemicals have been adopted globally as the relevant testing methods. OECD has been working to eliminate non-tariff barriers created by different regulations in different countries since the second half of the 1970s. It has set up the Chemical Programme that is concerned with the safety assessment of chemical compounds, under which standardization efforts are going on to consolidate the existing testing methods, for example, to determine the environmental fate of chemicals and the impacts on ecosystems and human health. As part of this program, a collection of in vitro tests have been developed to assess the ecological impacts of chemical compounds on aquatic and other organisms.

Particular emphasis is placed on the assessment of impacts on aquatic organisms, because many chemical compounds are believed to reach aquatic environments in the end and such environments are essential for human activities. This signifies the utmost importance of evaluating the impacts of chemical compounds on biota, which is indispensable for the conservation of aquatic environments.

3 Development of the OECD test guidelines for toxicity of chemicals

The OECD Test guidelines for toxicity of chemicals for the assessment of effects on biotic systems may be closely examined on the Web site, which are now being subject to additions and revisions to update. There are so far 17 approved guidelines for the tests, No. 201 to No. 217, as of March 2003. Table 1 shows the outlines of major tests adopted in the OECD guidelines.

OECD is making effort to develop and adopt new tests, as the existing tests alone do not provide sufficient assessment. The followings are major issues being examined at OECD.

3-1 Fish reproductivity / life cycle test

An introduction of a method for life cycle test in fish is being considered at OECD, because the existing tests do not include a chronic toxicity test. The life cycle test is intended to cover all life stages of fish from the newly fertilized eggs to the adult fish, and to define the effects of chemicals on reproductivity of test species (fitness). To evaluate the effects throughout the entire life stages of fish, Oryzias laptipes with a short life cycle has been made a candidate for test species.

3-2 Test for benthic organism

Benthic organisms such as crustacea and shellfish play a significant role in the purification of aquatic environments, as they consume organic matters in sediment. Residual water insoluble chemicals contaminated in aquatic environments tend to accumulate in sediment. Therefore the development of a test for species that live in sediment is becoming important, in addition to the tests for conventional swimming/floating species, such as algae, Daphnia magna and fish. New methods of the examination using juvenile Chironomid midge or Eisenia foetida. as test animals are now proposed and the investigations are under way.

3-3 Test for terrestrial organism

This test is intended, in large part, to determine the effects of agrochemicals on birds, honeybees and soil microorganisms. Fewer data have been available for terrestrial organisms compared to aquatic organisms. There are many tasks to be solved concerning the development of the tests for terrestrial organisms, including the revision of the existing tests.

3-4 Test for sparingly water soluble chemical substances

The OECD test guidelines for toxicity of chemicals are used to assess ecological risk in many countries around the world, which were focused mainly on the assessment of their effects on fish, Daphnia magna and algae in most cases. The tests for these aquatic organisms are based on years of discussions and accumulation of data and are known for its highly reliable reproducibility. However, they were originally intended for testing water-soluble substances. In reality, many of chemical compounds are sparingly water-soluble, which makes it difficult to conduct a test in many cases. Traditionally, co-solvent agents such as solvent and detergent have been added to test water to handle such substances.

In the 1990's, Europe took the lead in calling for a change in this practice to prevent interaction of test substances with auxiliary agents, which led to the establishment of the "Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures" in 2000. This guidance document declares that, in principle, no dispersing agent should be added to test water. The OECD test guideline for toxicity of chemicals is also moving towards the establishment of a principle in which exposure of test animals to a test substance should be carried out within the concentration of their water solubility without auxiliary substance.

Table 1 :Outline of major OECD test guidelines for toxicity of chemicals (Approved guidelines only)

No.	Test Name (Major Test Species)	Purpose of Test	Outline of Test Procedure
201	Alga, Growth Inhibition Test (Selenastrum capricornutum)	Assessment of effects on single-cell green algae as a primary producer in the biotic system.	Investigate the change in growth speed of algae by inoculating it into test culture containing a test article in the concentrations varying stepwise.
202	Daphnia sp. Acute Immobilization Test and Reproduction Test (Daphnia magna)	Assessment of acute toxicity effects on minute invertebrates as a primary consumer in the ecosystem.	Investigate the inhibition of the swimming (abnormal behavior) of juvenile Daphnia magna after 48 hour exposure to test water containing a test article in the concentrations varying stepwise.
211	Daphnia magna Reproduction Test (Daphnia magna)	Assessment of chronic effects on Daphnia magna as a primary consumer in the ecosystem. Examination of the numbers of adult Daphnia magna whose swimming was interrupted together with the numbers of their offspring during 21 days of feeding and exposure to test water containing a test article in the concentration varying stepwise after inoculating juvenile Daphnia magna to the water.	
203	Fish, Acute Toxicity Test (e.g., Oryzias latipes, Brachydanio rerio)	Assessment of acute toxicity effects on fish, representative species of aquatic organisms and are high order consumers in the ecosystem.	Determine the lethality of a test fish after 96 hour exposure to test water containing a test article in the concentration that changes stepwise.
204	Fish, Prolonged Toxicity Test (e.g., Oryzias latipes, Brachydanio rerio)	Applicable to substances having high lipid solubility and accumulate gradually within the fish body to express their toxicity.	Determine the lethality and the presence of abnormal behavior of a test fish during 14 day exposure to test water containing a test article in the concentration varying stepwise while feeding.
210	Fish, Early-Life Stage Toxicity Test (e.g., Oryzias latipes, Brachydanio rerio)	Determine if there is a lethal effect on fish in an early stage of life from embryo to juvenile fish.	Place fertilized eggs of the test fish to hatch. Determine the effect on the rate of hatching, survival of juvenile fish after hatching, and growth.
209	Activated Sludge, Respiration Inhibition Test	Determine the effects on activated sludge being a community of microorganisms responsible for decomposition in the biotic system.	Determine the change in respiratory activity by adding a test article to activated sludge from a sewage plant in a manner where concentration varies stepwise
205	Avian Dietary (Mixed Feeding) Toxicity Test(e.g., Anas platyrhynchos, Coturnix japonica)	Determine the extent of acute dietary toxicity on birds.	Determine the lethality by feeding for 5 days with bird feed containing a test article in the concentrations varying stepwise.
206	Avian Reproduction Test (e.g., Anas platyrhynchos, Colinus virginianus, Coturnix japonica)	Determine the effects on avian reproduction.	Feed a test bird for 8 weeks with bird feed containing a test article in the concentrations varying stepwise followed by having it lay eggs in the subsequent few weeks. Examining the death, weight, symptoms of parent birds, number of laid eggs, rate of hatched eggs, and the survival rate and weight of juvenile birds to determine the effects on avian reproduction.
207	Earthworm, Acute Toxicity Tests (Eisenia foetida)	Determine the effects on Eisenia foetida as a decomposer of soil.	Determine the lethal concentration by having Eisenia foetida contact with a filter paper impregnated with a test article in the concentrations varying stepwise.
203	Terrestrial Plants, Growth Test	Determine the effects on germination and early growth of terrestrial plants.	Control the soil artificially to contain a test article in the concentrations varying stepwise, grow test plant, followed by measuring the rate of germination and the weight of harvest to work out inhibitory concentration on germination and growth of the test organism.
213	Honeybees, Acute Oral Toxicity Test (Apis mellifera)	Assess acute oral toxicity to adult honeybees as a useful insect.	Give 100 to 200 μg of water containing 50% sucrose and an appropriate concentration of a test article to determine the lethality concentration.
214	Honeybees, Acute Contact Toxicity Test	Assess acute contact toxicity to adult honeybees as useful insects.	Apply, with a micro applicator, 1 ul of solution containing an appropriate concentration of a test determine the lethal concentration.

The ban on the use of dispersing agents is based, in part, on results of experiments showing that only "dissolved" test substances demonstrate bioavailability for test species, and that test substances remain un-dissolved in the form of "suspended materials", are hardly taken into the body of organisms. Test substances are taken into the body of aquatic organisms mainly through bio-membrane such as gills, a mechanism known as passive diffusion, and un-dissolved test substances are not supposed to interact with bio-membrane.

Test substances, while showing toxicity when they are dissolved, insoluble remainder of the substances can also physically exhibit their apparent toxicity. This happens when aggregates formed from un-dissolved substances that may stick to gills or bio-membrane, or may block up the respiratory organ or feeding organ, which could result in the inhibition of the swimming behavior or the death of test species, even if test substances themselves have no toxicity. Therefore, the use of dispersing agents should be avoided in order not to destroy the aggregates causing apparent toxicity, together with the fact that the use of dispersing agent is unrealistic since, in the natural field, the concentrations of the chemical substances in water never exceed their intrinsic solubility.

4 Testing methods adopted in the US and member states of the EC

As pioneers in the field of ecological risk assessment, the US and EU have developed and established various testing methods using many species. Their development activities exert a substantial influence on the development of new guidelines at OECD.

There are two major guidelines in the US, one developed by ASTM (American Society for Tests and Materials) and the other released by US-EPA (US Environmental Protection Agency). ASTM publishes standard test methods that enable the toxicity assessment of not only chemical substances but also of discharged water.

EPA issued two Test Guidelines, OTS (Office of Toxic Substances) Guidelines intended for industrial chemicals and OPP (Office of Pesticide Program) Guidelines for pesticide chemicals. OPPTS (Office of Prevention, Pesticide and Toxic Substances) has consolidated these guidelines to develop new test guidelines to ensure harmonization with the OECD test guidelines for toxicity of chemicals. Among the OPPTS Guidelines, the Ecologic Effects Test Guidelines called the Series 850 have been accessible through the EPA's Web site since 1996.

Table 2 Data set of ecological effect tests required for registration of new chemical substances by individual country

Country	US	Canada	EU	Japan
Production volume to be regulated	less than 10 tons per year	more than 10 tons per year	more than 1 ton per year per manufacturer	more than 1 ton per year
	Data in hand acceptable.			
Fish, Acute Toxicity	(required for certain circumstances)	○	○	○ *
Daphnia magna Acute Toxicity	(required for certain circumstances)	○	○	○ *
Algae, Growth Inhibition	(required for certain circumstances)	–	○	○ *
Activated Sludge, Respiration Inhibition	(required for certain circumstances)	–	○	–
Biodegradability	(required for certain circumstances)	○	○	○
Bioaccumulativity	(required for certain circumstances)	–	–	○
Hydrolyzability	(required for certain circumstances)	–	○	–
Adsorption-Desorption Screening	(required for certain circumstances)	–	○	–

* Required under the revised Chemical Substances Control Law.

In the meantime, the OECD test guidelines for toxicity of chemicals have been established as the standard guidelines in the EU, because the EU member states represent a large part of the OECD countries and the OECD guidelines originally derived from the EU guidelines.

The following testing methods are used to obtain data required for the toxicity assessment of new chemicals to be manufactured or of import chemicals to be registered. Table 2 shows testing requirements by countries.

Unlike other countries, there is no need to submit specific pre-manufacture data items when applying for the registration of new chemical substances in the US, because the data in hand are considered sufficient. Upon the receipt of application, EPA estimates the effects of chemicals based on Structure Activity Relationships (a technique routinely used by EPA to estimate physiological activity or toxicity of new chemicals from their chemical structure, using the database on a correlation between the structure and physiological activity of chemical compounds) and may request for the submission of relevant data.

In Japan, "Law Concerning the Evaluation of Chemical Substances and Regulation of their Manufacture, etc." (hereinafter "Chemical Substances Control Law") was applied to industrial chemicals to ensure their risk management. There was no need to submit data on an ecological toxicity test, because the purpose of this law was to evaluate the impacts of chemicals on human health through environmental pollution. However, an ecological toxicity test became a part of the screening requirements when the law was revised in May 2003.

5 Regulations and management of chemical compounds in Japan – revision of the chemical substances control law –

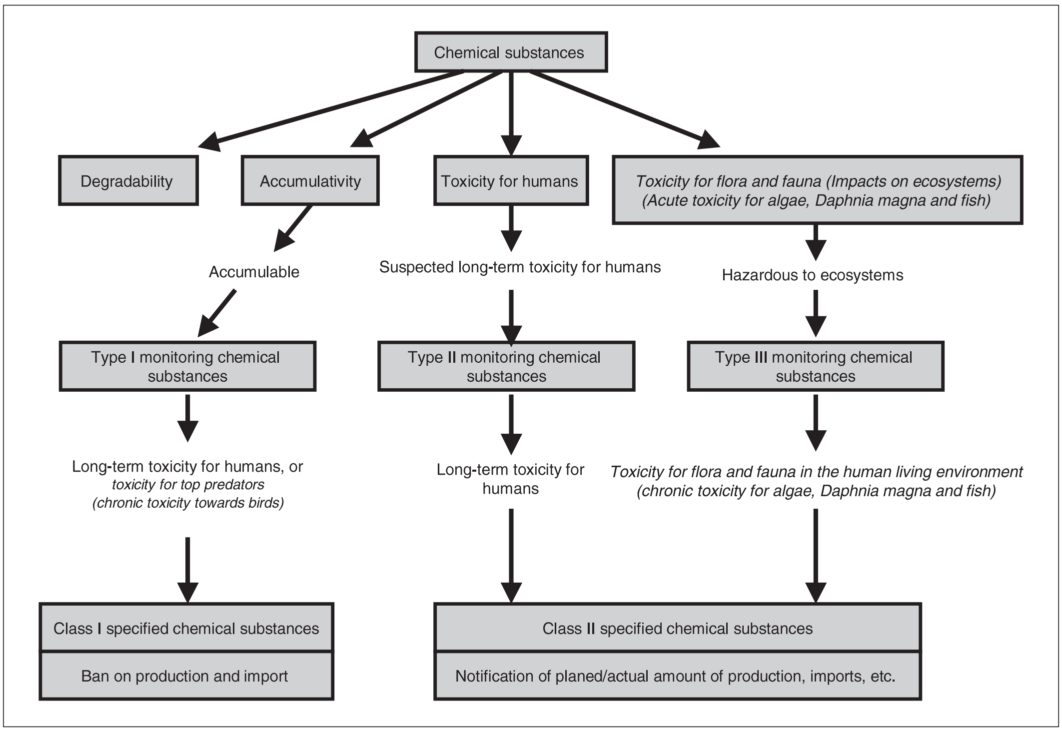
As already said, Japan had not taken any legislative measures to impose the assessment of ecological toxicity of chemical compounds, despite the efforts of other industrial nations to develop and implement measures for wildlife conservation. As such delay was clearly against global trends of the time, the OECD Environmental Performance Review of January 2002 recommended that Japan "further extend the scope of regulation to include ecological toxicity."

The Basic Environment Plan has "Harmonious Coexistence" as one of the long-term objectives and the National Biodiversity Strategy outlines the general direction of Japan's domestic environmental policies concerning chemical compounds. Both of them state the need to "facilitate the assessment and management of the effects on ecosystems in an appropriate manner" with regard to measures concerning chemical compounds. The result was the revision of the Chemical Substances Control Law. The revised law has a new objective to "prevent environmental contamination caused by chemical compounds that may impact human health and wildlife."

The revised law includes the ecological toxicity test as a screening requirement for the registration of new chemicals with an eye to identifying chemical compounds that may have some impact on ecosystems. Specifically, the acute toxicity test must be conducted for algae, Daphnia magna and fish (Oryzias latipes), representative species in the food chain, as is required in the US and Europe.

Figure 1 shows the outline of procedures required under the new screening system. Under the revised law, production and import volume will be monitored for chemical compounds toxic to animals and plants, even if there is no "long-term toxicity against human beings." If there is a risk of environmental pollution, employers will be directed to conduct the "chronic toxicity test" for animals and plants that are likely to be impacted as part of the hazard assessment. If that impact may extend to animals and plants that are related to the human environment (e.g., useful and familiar animals and plants), the employer is obliged to register the concerned chemical compounds as Class II Specified Chemical Compounds and notify of the scheduled production and import volume, in addition to the actual production and import

Figure 1 : Outline of screening and regulation systems for chemical substances under the revised Chemical Substances Control Law



Italics show new screening requirements adopted by the revised law.

volume. If necessary, measures will be taken to limit the synthesis and import volume of such chemical substances.

Production and import of “Class I Specified Chemical Compounds” such as PCBs, which are persistent environmental contaminants that accumulate rapidly in the human body and show long-term toxicity, are already banned. Under the revised law, chemical compounds toxic to animals such as birds and mammals high up in the food chain are also subject to similar regulations (even if they are not toxic to human beings).

All the ecological toxicity tests shall be done in accordance with the OECD Test Guidelines for Toxicity of Chemicals. Guidelines for the “Acute toxicity test for Algae, Daphnia Magna and Fish,” which are necessary for the preliminary review of new chemical compounds, are almost ready. Presently, the law envisages introducing the “Chronic Toxicity Test for Algae, Daphnia Magna and Fish” as a means to examine “toxicity against familiar animals and plants” for the classification of new chemical compounds as “Class II Specified Chemical Compounds.” Furthermore, the “Test for Avian Reproduction” is expected to be adopted for the assessment of “Toxicity against High-level Predators” to classify new chemical compounds as “Class I Specified Chemical Compounds.”

Japan has little experience in conducting the chronic toxicity test or reproduction test, which makes it necessary to increase the number of laboratories that comply with Good Laboratory Practice (GLP) in the future.

6 Definition of sparingly soluble chemical substances in the revised Chemical Substances Control Law
– consistency with global economy –

In connection with the inclusion of the ecological risk assessment in the Chemical Substances Control Law, there was a heated discussion on how to address the issue of “sparingly water soluble substances.” As already stated, the existing OECD test guidelines for toxicity of chemicals provide that no dispersing agents should be added to test water when conducting a test for sparingly water soluble chemical compounds and that their concentrations should never exceed the maximum concentration in purified water, namely the aqueous solubility. However, chemical compounds of strong physiological activity tend to have high lipid solubility (e.g., insecticide, fungicide), hence sparingly water-soluble. The law provides that all toxicity tests shall be done at the concentration within the water solubility of the chemicals. So, there is a possibility that it would be wrongly concluded that compounds hard to dissolve “pose no threats to ecosystems.”

As the OECD members, the US and EU have long started efforts to develop tests to deal with the issue of sparingly water soluble chemical substances, based on the understanding that they tend to move into sediment. Examination is under way to establish the test for benthic organisms, using Chironomid midge in the form of embryo and other organisms. If this benthic organism assessment is formally adopted as the OECD test guidelines for toxicity of chemicals, Japan will introduce it in the future. This, however, raises a question. Can we afford to leave the issue of sparingly water soluble chemical substances unaddressed until such time as the said testing method is approved, knowing that Japan relies more heavily on water resources including rivers and lakes than other countries? In the process of revising the law, the author pointed out this problem as a member of the Ministry of Environment Ecological Risk Test and Assessment Technology Commission. The author then proposed the use of detergent and other agents to some extent to observe how sparingly water soluble substances demonstrate their activity against aquatic organisms when the aqueous solubility cannot be determined. The ultimate objective of the ecological toxicity test is “wildlife conservation,” although it uses only three aquatic organisms, namely algae, Daphnia magna and fish, as test species. This prompted the author to decide that some options should be introduced to learn as much as we can from these tests. For this proposal to be adopted into the revised law, the potential creation of trade barriers posed the biggest problem. Because the use of auxiliary agents do not comply with the OECD guidelines, it is not possible to request importers of new chemical substances to conduct the test using auxiliary agents, or to submit test data involving the use of auxiliary agents for export purpose. After endless discussions, a draft of the ministerial ordinance was developed. It stipulates that the law can demand a test that uses the dispersal system and auxiliary and other agents, provided that it is impossible to measure the aqueous solubility and obtain acute toxicity data concerning aquatic organisms. This example shows that the global free market could be a cause of new screening requirements.

7 Need for and direction of new ecological risk assessment

Japan will at long last join other industrial nations as it begins the ecological risk assessment of chemical compounds. However, the assessment is based sorely on toxicity tests for a few test species adopted in line with the international standards, which created more than a little criticism that such assessment is absolutely insufficient for the protection of the ecosystem as a whole.

An ecosystem is a complex organic network consisting of a dynamic set of living organisms all interacting among themselves. Different ecosystems are found in different regions, and, as already stated, there is an immense number of species on the earth that are crucial to its biodiversity. It should not be a surprise if some question the relevance of relying on the toxicity test conducted in a beaker for only three species (algae, Daphnia magna and fish) to protect such complex and diversified ecosystems.

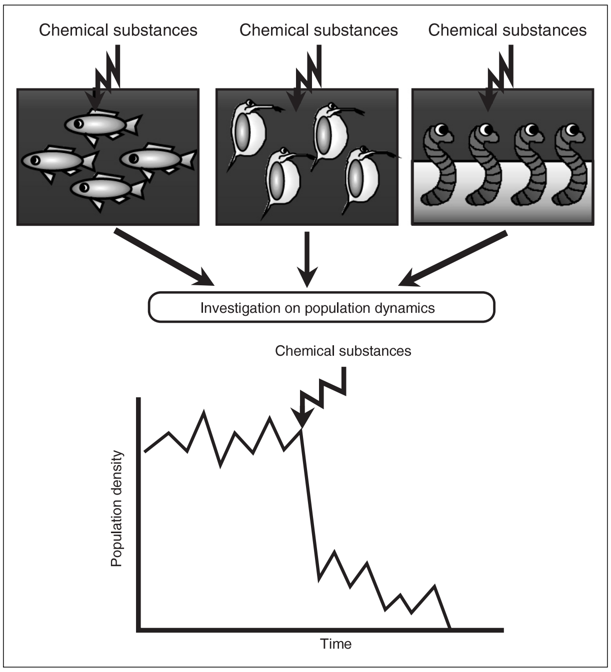
To respond to such criticism from the perspective of ecological science, continuing efforts are under way to explore ways to migrate from the risk assessment at an individual organism level to one at a higher level. For example, the US and Europe have proposed the use of more than five test species for a fish toxicity test, and developed a test at a population level. In addition, model development is being conducted to identify the effects of chemical compounds for each development stage of test species, work out how those chemicals impact the reproductivity (fitness) of test species, and finally estimate probability of extinction of the population (Figure 2).

A macrocosm test*1 provides a new possibility for the assessment of effects of chemical compounds at the ecosystem level (Figure 3). In this test, an "experimental ecosystem" is constructed to simulate parts of natural aquatic ecosystems by placing various species in a large tank set up in a laboratory or conservatory to which test substances are applied. These "high-level test systems" are aimed at assessing ecological effects of chemical compounds more "realistically" by reproducing a complex ecosystem in the best possible way to simulate a natural ecosystem. It is, however, simply impracticable to cover all natural ecosystems with different diversity in different regions or for different seasons, however complex the constructed test systems are. Any tests could end up a case study.

Meanwhile, development efforts are going on for genetic toxicity tests that do not use individual species. Toxicogenomics is a recently developed field in toxicology and involves a process known as microarray analysis, which obtains substantial quantities of data to identify the level of expression for all genes. It holds a key to estimating the toxicity of chemical substances towards living organisms as it identifies their effects on the expression of specific genes. This new technique, if established, is expected to lead to significant reductions in the time and costs associated with the breeding of test species and the standardization of tests, and to improve the efficiency of ecological effects assessment. It is necessary to investigate this technique more carefully to decide if risks to ecosystems can really be estimated by observing changes in gene expression.

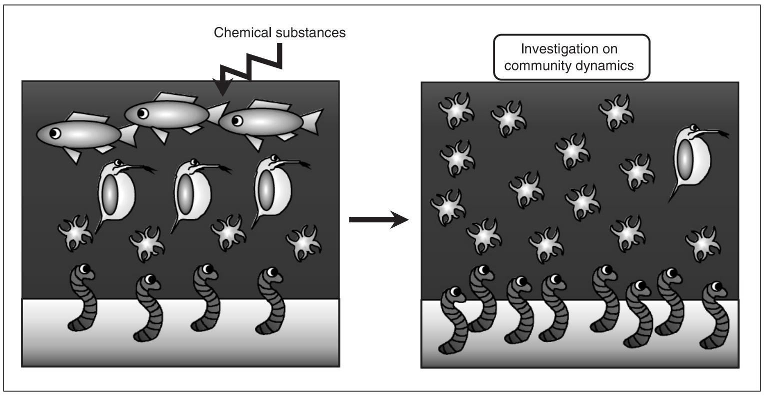
Rather, future ecological risk studies would require model development and validation studies to fill a gap between in vitro tests and natural field ecosystems. Toxicology must investigate ways to estimate potential events in natural ecosystems based on in vitro toxicity tests including those based on gene expression. To do so, it is necessary to examine the various possibilities from the standpoints of population biology such as population ecology, community ecology, and population genetics and to develop

Figure 2 : Ecological effects test in population level



Expose test population of test species to chemical substances and identify changes in population density.

Figure 3 : Ecological effects test in community level



Create a test community consisting of various species such as Chironomid midge, algae, Daphnia magna and fish, administered chemical substances to the community, and identify changes in the community structure (the formation of species and community density).

a mathematical model. The aforementioned "high-level test systems" should be used to accumulate data to confirm the effectiveness of a mathematical model. Data collection should also be conducted in various field locations.

8 Requirements for ecological risk assessment in Japan

It will take considerable time for such an ideal model to be established. A weak research environment and lack of researchers are two major obstacles for the advancement of ecological risk studies in Japan. It has been quite some time since words such as "conservation biology" and "biodiversity conservation" gained popularity. However, only a small number of ecologists address issues related to chemical compounds and their production and disposal. For example, the Japanese Society of Environmental Toxicology has only about 400 members, when its US counterpart SETAC (The Society of Environmental Toxicology and Chemistry), a leading society that represents eco-toxicology researchers in the US, has more than 5,000 members. These figures show that the Japanese researcher population in the field of environmental toxicity or ecological risk is smaller than that of the US and Europe. There is one other factor. In Japan, only a handful of university laboratories and research institutes are specialized in the assessment of ecological effects of chemical substances, and this makes it difficult to foster a large number of researchers. The immediate task required for Japan's ecological risk studies is to foster more biology researchers in a way so that they develop interests in environmentology and other research fields. To accomplish this, it is necessary to resolve a disjunction between the academic emphases of the societies for toxicology and the societies for ecology by developing a linkage mechanism that connects academic societies. The possibility of establishing a multi-disciplinary organization such as a consortium of academic societies should also be examined.

For the progress of ecological risk studies, it is necessary to create a social setting that would generate more attention and interest in this research field in general. Various possibilities should be looked into to promote the understanding of ecological risk assessment among the general public. Attaching labels with the words "Creature Friendly" to chemical compounds that meet the requirements of the ecological risk assessment may be a good idea. Chemical makers and distributors tend to see the ecological risk assessment as a heavy burden because it could lead to strengthened regulations. At the same time, it would enable them to publicly announce the safety of chemical compounds and differentiate them from other products, once they are confirmed complying with regulations. Environmentally friendly chemical compounds may help create a new market sector. If the ecological risk assessment provides some potential commercial benefit and more companies show interest in this research field, it will become a new, attractive research theme, which could grow into one research field in the future.

The OECD test guidelines for toxicity of chemicals and the Chemical Substances Control Law were derived in the shadow of two concepts, economic development and environmental conservation, which are contradicting but necessary for human society. The latest revision of the Chemical Substances Control Law and the inclusion of the ecological risk assessment represent a huge step forward. There is no doubt that requirements and criteria for the ecological risk assessment will be increasingly severe both domestically and internationally. As a big trader, Japan must be prepared to take the leadership in this field. The environmental effects assessment of chemical substances is essential for environmental conservation. Researchers with a love for nature should take a strong interest in chemical compounds, which are a near-cause of environmental destruction. Japan must strengthen its efforts for the advancement of ecological risk assessment studies.

Glossary

*1 Macrocosm test

The word macrocosm, derived from a "miniature universe," means an artificial ecosystem created in a tank in which a population or community of organisms are cultivated. In short, it is a flask level test system conducted in a controlled environment. For comparison, a large-scale test system conducted in the enclosing parts of existing ecosystems is called the mesocosm test.

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