Ecotoxicological Risk Assessment of Pesticides in Aquatic Ecosystems

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Ecotoxicological risk assessment of pesticides in aquatic ecosystems has become one of the most important areas of scientific pesticide evaluation. Sumitomo Chemical has been developing many pesticides in order to maintain a stable worldwide food supply, and we have been conducting high quality ecological risk assessments by using state-of-the-art techniques for the evaluation of our pesticides. In this article, the outline of the aquatic ecological risk assessment procedures in Japan, the USA and the EU are briefly summarized and some examples of sophisticated higher-tier ecotoxicological studies undertaken to demonstrate that our pesticides are benign to aquatic environment are introduced.

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Introduction

With the goal of providing food for the human race as well as having a stable food supply at present and in the future, agricultural chemicals (i.e. pesticides) are being disseminated as economically effective methods for preventing reduced yields in crops due to damage by insects and weeds. The pesticides are roughly divided from their targets into insecticides which include insect growth regulators for exterminating harmful insects, fungicides for preventing and eliminating fungi such as powdery mildew affecting the growth of plants, and herbicides which include plant growth regulators for preventing weeds, and each of them has a superior efficacy for organisms that are harmful to crops. These pesticides are intentionally applied outdoors to agricultural land, so in addition to the efficacy of pesticides for various diseases and insects that damage crops (benefit), evaluations of their impact not only on the health of farmers and consumers but also wild animals (risk) are necessary in determining their usefulness as pesticides. There is a long history of safety evaluations based on various types of toxicity tests using mammals from the standpoint of assuring the health of humans, but there has been an increase in the awareness of environmental protection recently, and evaluations of the effects of pesticides on the environment, including wild animals, have become indispensable for development of new pesticides and maintaining the registration of existing chemicals already on the market. However, there is a wide variety of wild animal species to be evaluated, and just among the vertebrates alone, there are various types including mammals, birds, reptiles, amphibians and fish. In addition, the diversity of their ecology and shortage of information on life history of some species make it very difficult to evaluate the safety for individual species. Incidentally, since the pesticides that are applied to cultivated land are considered to enter the hydrosphere of the rivers and lakes adjacent to the cultivated fields with the movement of air and rain as they undergo various types of metabolism and decomposition, the evaluation of the environmental impacts of pesticides on the ecosystem of the hydrosphere is one of the most important areas in evaluations of safety. Because of the complexity in ecology of the organisms living in the aquatic ecosystem as part of the food chain, the remarkable regional characteristics in ecosystems as observed for the differences in habitat and ecosystem between the Great Lakes in the United States and Lake Biwa in Japan and, in addition, the different aquatic ecosystem to be protected depending on the culture, ideologies and values of the people in various regions, etc., it is not only difficult to make uniform assessments, but also the current situation is one in which there is a great variety in the methodology for evaluation taken by the agencies regulating pesticides in various countries.

Sumitomo Chemical has been developing many pesticides in order to maintain a stable worldwide food supply, and we have been conducting high quality ecological risk assessment by using state-of-the-art techniques for the evaluation of our pesticides. In this article, the outline of the aquatic ecological risk assessment procedures in Japan, the USA and the EU are briefly summarized and some examples of sophisticated higher-tier ecological studies undertaken to demonstrate that our pesticides are benign to aquatic environments are introduced.

Environmental Assessments in the Hydrosphere

1. Concept of assessing environmental impact

In addition to first describing the variety and characteristics of the “natural environment” and “organisms” that are the target of assessments of environmental impact, we will give an overview of the “environmental behavior of pesticides,” which is important for knowing how organisms we are targeting are exposed to pesticides or their degradation products and metabolites generated in the natural environment. We will give a simple introduction to the basic concept of environmental assessment based on these.

(1) Natural environment and organisms

The natural environment, which is roughly divided into the “hydrosphere,” “pedosphere (soil)” and “atmosphere,” is formed of various ecosystems that interact with each other in a complicated manner. According to the Biodiversity Protocol,[1] an ecosystem is “a dynamic composite that forms a single functional unit where a community of plants, animals and microorganisms and the abiotic environment surrounding them interact”. In other words, the ecosystems we are targeting are ones in which there are a wealth of dynamic spatio-temporal changes due to physical (sunlight, water temperature, etc.), chemical (nutrients, trace metals, etc.) and geographical (climate, topography, etc.) environmental factors intimately linked in the complex interactions of the predation, prey, competition, parasitism, propagation, decomposition, etc., of the various organisms. As can be seen from the example of a schematic diagram of an ecological pyramid of an aquatic community shown in **Fig. 1**, a food chain, hierarchical (pyramid) structure and cycle of matter are established. The interaction of the predation, prey, competition, etc., of producers such as algae and a hierarchy of consumers, which are known as crustaceans and fish (primary consumers, secondary consumers, higher predators, etc.) and decomposers such as bacteria is woven here.

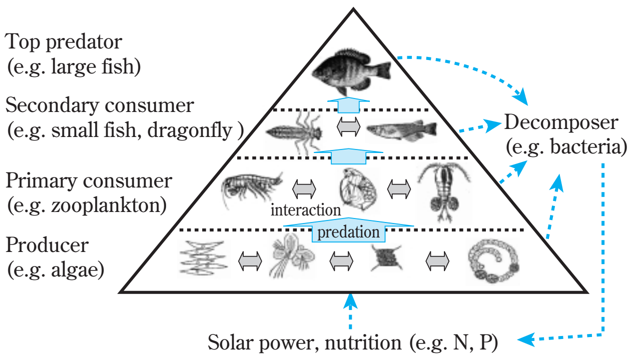


Fig. 1 Typical ecological pyramid in an aquatic community

Furthermore, the number of species on the earth, just with the presence of animals, is very large at one million or more,[2] and along with their being classified in detail as in **Table 1**, there is a great variety in habitats, lifecycles, lifestyles and reproductive strategies in each species. For example, in terms of the mode of reproduction, there is a division into asexual reproduction such as budding and division and sexual reproduction, and sexual reproduction may further divided into

Table 1 Taxonomic classification and variety of reproductive strategies of animals

Taxonomic class	Number of Species	Reproductive Strategy			
		Asexual	Sexual		
			Parthenogenetic	Bisexual	
				Ambisexual	Diecious
Invertebrate	999954				
Porifera	5000	○	×	○	Minor
Platyhelminthes	15000	○	○	○	×
Nemertinea	750	○	×	Minor	○
Aschelminthes	6985	×	○	×	○
Mollusca	112000	×	×	Minor	○
Annelida	7000	○	○	Minor	○
Tardigrada	280	×	○	×	○
Arthropoda	800000	×	○	○	○
Sipunculoidea	275	×	×	×	○
Hemichordata	100	○	×	×	○
Echinodermata	5970	×	×	×	○
Protochordata	1613	○	×	○	○
Other invertebrates	44981				
Vertebrate	43150	×	Minor	Minor	○

bisexual and parthenogenesis such as that for water fleas and rotifers not accompanied by both sexes. In addition, among the modes of bisexual reproduction, there are ambisexual forms such as snails and earthworms and diecious forms such as humans, birds and amphibians. In addition, the sex reversals undergone by some species of fish according to growth and social order and those according to incubation temperature in amphibians and reptiles are known (Table 1).[3] These are strategies for preserving the species and prospering in a harsh natural environment that is under various types of biological, chemical and physical stress.

(2) Environmental behavior of pesticides

Pesticides sprayed and applied to agricultural land exhibit complex, divergent environmental behavior, according to environmental factors in the surroundings and the physio-chemical properties of the pesticides as well as formulations as shown in the schematic diagram in **Fig. 2**.

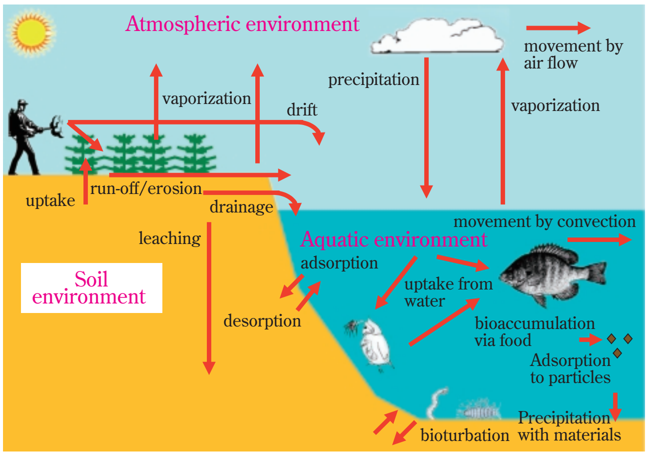


Fig. 2 Environmental fate of pesticides

For example, while most of the pesticides reach the crop and soil when they are sprayed, a small part may be drifted into the environment surrounding the agricultural land, including the hydrosphere because of diffusion and the flow of the air. In addition, the pesticides that attach themselves to the crop and soil also reach the aquatic system that is the target of the assessment of environmental impact via runoff, erosion and drainage from the agricultural land through volatilization into air and the downward movement by rainfall with repeated absorption and desorption in soil-water matrices (leaching). In addition, it can be assumed that, the concentration of pesticides in water changes dynamically because of the adsorption and desorption between water and the various environmental components such as sediment consisting of mud and various dead organisms deposited on the bottom of lakes and marshes, algae, microorganisms, dissolved organic matter like humic substances, and inorganic compounds such as suspended clay minerals. Along with the physical movement of these, pesticides are biotically metabolized by microbes in soil and natural water and by chemical reactions[4)–6)] such as hydrolysis and sunlight photolysis during the various processes of movement. In this manner, pesticides exhibit a variety of movement, metabolism and degradation behavior in the natural environment, and the dynamics of pesticides in the hydrosphere, and further, assessment of the effects on aquatic ecosystems are viewed as important because water is an important medium.

(3) Basic methods for assessing impact on ecosystems

We understand that both organisms in the natural environment and the environmental behavior of pesticides greatly depend on the area being evaluated in this manner, but risk-based evaluation methods that compare the potential of life being impacted for each target organism and the concentration to which organisms are exposed are fundamental. Risk assessments are not normally done in a single stage, but usually progress in a so-called tiered system that increases in refinement in a direction where the ecological effects and extent of exposure are closer to the natural environment in multiple stages as shown in **Fig. 3**.

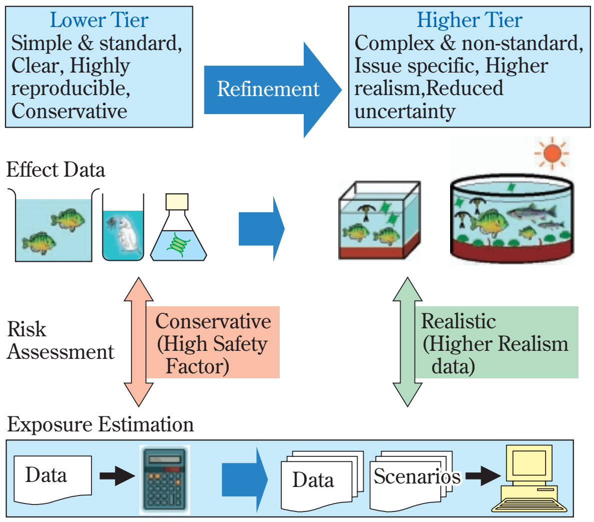


Fig. 3 General principle of the ecological risk assessment scheme

In the initial lower tier, assessments are carried out with conservative safety coefficients determined by each regulatory agency by comparing the ratios of predicted concentrations in the environment calculated by simple exposure estimation methods and index values for acute ecological impact (normally exposure within one week) and long-term ecological impact (normally exposure from several weeks to several months) obtained in the laboratory using standardized test methods such as determined by the Organization for Economic Cooperation and Development (OECD) and the U.S. Environmental Protection Agency (EPA) for representative standard organisms such as rainbow trout and bluegill for fish (higher trophic level consumers), water fleas for invertebrates (lower trophic level consumers) and green algae for plants (producers). When sufficient safety is not secured by this conservative method, higher tier risk assessments are carried out. There are a great variety of test systems in higher tier assessments, including various simulated ecosystems from small scale ones such as a model ecosystem[7] simulating a small natural world in indoor aquaria to large scale ones that are implemented outdoors. Data on the ecological effects under conditions simulating the natural world is obtained by setting up a complex test system that envisions points of concern in the assessment of ecological risk and the aspects of exposure in the natural environment to make more refined assessments. On the other hand, highly precise calculations of the concentration predicted in the environment are made using various environmental behavior parameters. Moreover, in assessments using data obtained in a form closer to the natural world, the safety factor (10, for example) set conservatively in lower tier assessments is typically reduced (1, for example) in consideration of reducing uncertainty factors.[8]

In actual lower tier assessments, median lethal concentration (LC_{50}), median effect concentration (EC_{50}) and no-observed-effect concentration (NOEC) are used as index values for standard ecological effects, and factors such as organic carbon normalized adsorption coefficient (Koc) expressing the extent of partition to soil or sediment, disappearance time 50 (DT_{50}) as a dissipation index are used for estimating the exposure concentrations. On the other hand, the assessment values in higher tier tests for assessments of environmental effects differ according to the method, and for example, with test systems using simulated ecosystems, the no-observed-ecologically-adverse-effect concentration (NOEAEC), which takes into consideration the effects that are ecologically permissible and recovery properties from the symptoms of effects, is used.

2. Aquatic Risk Assessments in Various Regions (Japan, United States and EU)

Environmental risk assessments for the pesticide registrations vary in each country and region, and as an example, we will introduce the methods for assessing the risks to aquatic ecosystems in fresh water in Japan, the United States and the EU.

(1) Japan

The data requirements, assessment methods and acceptable standards[9)–11)] for Japan are given in **Table 2**. In Japan, assessments that place importance on acute effects where short-term exposure is envisioned in comparatively steep rivers that are representative of the fresh water are characteristic.

Table 2 Data requirements and aquatic ecotoxicological risk assessment in Japanese pesticide registration

	Lower Tier	Higher Tier
Effect	Acute/Short-term LC_{50}/EC_{50} Fish : Carp or Medaka, 96h Invertebrate : *Daphnia magna*, 48h Aquatic plant : Green alga, 72h Chronic/Long-term NOEC Invertebrate*: *Daphnia magna*, 21d	Additional species test (2-6 species) Lowest $L(E)C_{50}$ Bioavailability in natural water $L(E)C_{50}$ at TOC1.5mg/L Life stage (adult/neonate) sensitivity Geometric mean $L(E)C_{50}$
Exposure	Tier 1 Simulation PEC Input parameter : Use pattern	Tier 2/3 Simulation PEC Input parameter : Use pattern, Chemical properties (e.g. measured concentration), Scenarios (e.g. water flow)
Risk Assessment	Comparison of AEC and PEC AEC = fish LC_{50}/10, *Daphnia* EC_{50}/10, algal EC_{50}/1	Comparison of AEC and PEC AEC = lowest $L(E)C_{50}$/(2-4), $L(E)C_{50}$ at TOC1.5mg/L, Geometric mean $L(E)C_{50}$

* : Conditionally required (trigged by pesticide profile)

In lower tier evaluations, acute (or short-term) test data on fish (carp or killifish), *Daphnia magna* and green algae (*Pseudokirchneriella subcapitata*) are required, and in terms of long-term tests, data on the reproductive effects on *Daphnia magna* for insect growth regulators having a long persistency in water are required.[9)] Risk assessments are carried out by calculating the acute effect concentrations (AEC) by dividing the LC_{50} and EC_{50} values obtained in acute or short-term tests by the uncertainty coefficient, that is, 10 for fish and water fleas and 1 for green algae and comparing the minimum value with the predicted environmental concentration in water (PEC). When AEC is lower than PEC and there is any concern about the ecological impact, the precision of AEC is increased by reassessments that are closer to reality than the ecological effects obtained in lower tier tests using higher tier tests focusing on differences in sensitivity among species and growth stages and bioavailability of the pesticides affected by the dissolved humic substances in natural water. In parallel with this, the precision of PEC is increased from the tier 1 value numerically calculated based on an application rate to a tier 2 one incorporating decline of a pesticide in paddy fields and its outflow from non-paddy fields, and a tier 3 one which takes into account actual field data. Moreover, flow-through microcosm tests close to the actual environment, recovery tests, etc. are cited as future issues for higher tier assessment methods. [10), 11)]

(2) United States

The data requirements, assessment methods and acceptable standards[12)–18)] for the United States EPA are given in **Table 3**. In lower tier assessments, the point of multi-stage test requirements according to various triggers can be said to be one of the characteristics.

In lower tier assessments, acute (or short-term) tests from two species of fish (rainbow trout and bluegill as a general rule), *Daphnia magna*, and green algae (*P. subcapitata*) are required, and depending on the application, data for duckweed and alga (diatom, blue-green algae) other than green algae is required. In addition, early life stage (ELS) toxicity tests that examine the effects of exposure from fish eggs to fry and reproduction test on *Daphnia magna* are required for long-term tests.[12)] The risk quotients (RQ: EEC/LC_{50}, EEC/EC_{50}, EEC/NOEC), which are ratios of the estimated environmental concentration (EEC) to the short-term LC_{50} and EC_{50} and the long-term NOEC, are calculated. To ensure safety, in the acute and the long-term (including alga and duckweed tests evaluating growth), these must be less than 0.1 (0.05 for endangered species) and 1. When this is insufficient, detailed risk assessments are implemented using higher tier evaluations, (more precise EEC and higher tier ecological effect assessment test data), or changes in application techniques (risk mitigation), such as reduction of an application rate or setting up of a buffer zone, are required.[13)–15)] By the way, the more complex standard tests for evaluating ecological effects of the second tier become necessary depending on the outcome of the lower tier tests for evaluating ecological effects and the results of environmental fate tests. For example, fish full life cycle (FLC) toxicity tests become necessary depending on the results of ELS tests, but short-term or long-term tests with sediment dwellers such as midges and amphipods become necessary when pesticides are predicted to persist remarkably in the bottom sediment from their physio-chemical properties and environmental fate.[12)] The RQ values are evaluated as above, and if necessary, higher tier assessments are carried out for safety evaluations.

In terms of higher tier tests for assessing ecological effects, we can cite outdoor mesocosm experiments on a scale of 0.1 hectares, but because of the complexity of the experiment and difficulty of interpreting the biological results by advanced statistical methods, EPA has not as a rule require the tests recently. There have been proposals for mesocosm experiments on a smaller scale, for risk-benefit analysis and risk management instead.[13)–16)] Additionally, in terms of EEC calculated in parallel, precision can be increased using the EXAMS-PRZM exposure simulation shell (EXPRESS),[17), 18)] which is a higher tier assessment

Table 3 Data requirements and aquatic ecotoxicological risk assessment in US EPA pesticide registration

	Lower Tier	Higher Tier
Effect	Acute/Short-term LC_{50}/EC_{50} Fish : Rainbow trout and Bluegill, 96h Invertebrate : *Daphnia magna*, 48h ; Midge*, 10d ; Amphipod*, 10d Aquatic plant : Green alga, 96h ; Other plants* (Blue-green alga, Diatom, 96h ; Duckweed, 7d) Chronic/Long-term NOEC Fish : ELS test, FLC test* Invertebrate : *Daphnia magna*, 21d ; Midge*, 64d ; Amphipod*, 42d	Simulated or actual field test (possibly including microcosm test)
Exposure	GENEEC2 Simulation EEC Input parameter : Use pattern, Chemical properties (e.g. Koc)	EXPRESS Simulation EEC Input parameter : Use pattern, Chemical properties (e.g. Koc), Scenarios (e.g. meteorological)
Risk Assessment	Risk quotient evaluation acute RQ = EEC/L(E)C_{50} < 0.1 chronic RQ = EEC/NOEC(plant EC_{50}) < 1	Case by case

* : Conditionally required (trigged by use pattern, physico-chemical properties, other effect data, etc.)

simulation based on factors such as physio-chemical properties, environmental fate, growing conditions for crops, the physical properties of the soil, and long-term climate data from tier 1 simulations using the GENeric Estimated Environmental Concentration model, version 2 (GENEEC2).[17] Moreover, we can also cite methods, such as population modeling and precise exposure assessment, that make use of the Geographic Information System (GIS) based on detailed geographical information or more probabilistic measures in the report[19] by the Ecological Committee on FIFRA Risk Assessment (ECOFRAM), which has examined assessment methods for the future.

(3) Europe (European Union)

The data requirements, assessment methods and acceptable standards[20)–23)] for the EU are given in **Table 4**.

Table 4 Data requirements and aquatic ecotoxicological risk assessment in EU pesticide registration

	Lower Tier	Higher Tier
Effect	Acute/Short-term LC_{50}/EC_{50} Fish : Rainbow trout & 1 fish, 96h Invertebrate: *Daphnia magna*, 48h ; Midge*, 48h ; Other species* Aquatic plant: Green alga, 72h ; Other plants* (Blue-green alga or Diatom, 72h ; Duckweed, 7d) Chronic/Long-term NOEC Fish : Prolong or ELS or FLC test Invertebrate : *Daphnia magna*, 21d ; Midge*, 28d ; Other species*	Microcosm/Mesocosm Modified exposure test Indoor multi-species test Outdoor multi-species test Species Sensitivity analysis Additional species tests Probabilistic approach
Exposure	FOCUS STEP 1/2 Simulation PEC Input parameter : Use pattern, Chemical properties (e.g. Koc)	FOCUS STEP 3/4 Simulation PEC Input parameter : Use pattern, Chemical properties (e.g. Koc), Scenarios (e.g. meteorological)
Risk Assessment	TER evaluation TER_{st} = L(E)C_{50}/PEC>100 TER_{lt} = NOEC (plant EC_{50})/PEC>10	Case by case

* : Conditionally required (trigged by use pattern, physico-chemical properties, other effect data, etc.)

In lower tier assessments, acute (or short-term) tests from two species of fish (rainbow trout and one species of warm water fish), *Daphnia magna*, and green algae (preferably *P. subcapitata* or *Scenedesmus subspicatus*) are required, and depending on the application, data for duckweed, algae other than green algae and midges are required. In addition, when it is impossible to deny long-term or repeated exposure of the targeted species depending on the application method of a pesticide, long-term tests on fish and reproduction test on *Daphnia magna* are required.[20)] The toxicity exposure ratio (TER value: LC_{50}/PEC, EC_{50}/PEC and NOEC/PEC), which is the ratio of LC_{50}, EC_{50} and NOEC to the predicted environmental concentration (PEC) is calculated, and to assure safety, acute and long-term (including alga and duckweed tests) must be greater than 100 and 10, respectively.

If the TER value is insufficient, higher tier tests and safety assessments based on points of concern in the ecological effects are required. In addition, when a remarkable persistence of a pesticide in the bottom sediment is predicted from the profiles of environmental fate, long-term tests on benthic organisms such as midges are required, and assessments using the TER values as described above and higher tier assessments are required.[21), 22)]

In higher tier assessment, a reduction of uncertainty factor (TER standards: acute 100 and long-term 10) is recommended by obtaining indices for multiple species as well as the standard tests under water-sediment conditions simulating exposure in the natural environment, and indoor multi-species microcosms and outdoor multi-species mesocosms where the biodiversity and interactions among various species can be evaluated. Recently, probabilistic approaches increasing precision in assessment through sensitivity distributions for various nonstandard species, etc., can be cited.[22)] In terms of predicted concentrations in the environment, which are the exposure concentrations, stepwise simulations (FOCUS surface water) have been made mandatory by the Forum for the Coordination of Pesticide Fate Models and their Use (FOCUS), and starting with Step 1 and 2 where conservative exposure concentrations are calculated with multiple applications in southern and northern EU regions under the unrealistic worst-case scenario, and then Step 3 and 4, where more realistic simulations that integrate the climate for each crop and geographical conditions are carried out. In Step 1 and 2 calculations are made based on the physio-chemical properties of a pesticide and environmental behavior parameters and other simple parameters, but in the higher tier stages, refined simulations based on a large amount of physio-chemical properties, environmental fate data, data related to the growth of crops, specific climate and geographical data, etc., and further reduction of drift by setting up a buffer zone between the agricultural land and hydrosphere as a measure for reducing exposure are envisioned, and a remarkable refining of the PEC is possible.[23] Moreover, in the report[24] put out in 2005 by the FOCUS Working Group, which was aimed at further methods for assessment in the future and more advancement of measures for reducing impacts, there were proposals for meta-population modeling as a method for refined evaluations of recovery properties for ecosystems based on the migration from the vicinity of the populations that are affected in addition to various methods for reducing exposure and increasing precision, such as reduction of drift using drift-less nozzles and consideration of reduction of the flow into hydrosphere due to runoff and erosion using buffer zones.

Specific Examples of Higher Tier Tests in Assessments of Ecological Effects

1. Characteristics of higher tier studies

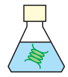
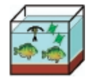
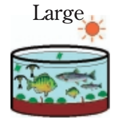
There is no fixed form for the test design of higher tier studies, and they are carried out case-by-case with consideration given to points of concern in the ecological effects on the targeted species and refined evaluations under conditions closer to reality. For example, we can cite the accumulation of standard test data with additional species and differences (between species and between growth stages) in the sensitivity of organisms using probabilistic analysis, but in terms of test methods carried out for various types of pesticides, there are simulated ecosystem studies that perform assessments of ecological effects directly on the real environment, that is, microcosm and mesocosm studies.

From the beginning, microcosm and mesocosm studies have been used as effective research methods for assessing the effects of chemical, physical and biological stress on ecosystems, without being limited to pesticides.[25), 26)] They are thought of as effective test methods for higher tier studies of pesticides because of the possibility for evaluating not only the environmental fate including movement and degradation but also interactions between a wide variety of organisms as shown in Fig. 1.

By the way, microcosm and mesocosm do not have clearly separated definitions, and generally, they are often distinguished by differences in scale and in being outdoor or indoor.[27), 28)]

Table 5 gives a summary of the comparison of the features of standard laboratory tests (lower tier studies), microcosm studies and mesocosm studies.

Table 5 Comparison of the standard lab test, mesocosm and microcosm studies

	Lab test	*Microcosm test*	*Mesocosm test*
Size of the test system	Small 	Medium 	Large 
Uncertainty of risk assessment	High		Low
Ecological and environmental reality	Low		High
Interaction of species	Few		Many
Repeatability and handling	High		Low
Volume of effect information	Low		High
Complexity of interpretation	Low		High
Cost and work volume	Low		High

Since, in general, the larger the system is, the more species, environmental factors and food chains are included, and the realistic nature of the ecosystem increases. Not only direct ecological effects, but also more refined evaluations of indirect effects (for example, drops in reproductive numbers because of reduction in food organisms) on insusceptible organisms caused by the interactions of organisms and community structures of the ecosystem as a whole can be assessed with a greater precision. On the other hand, the larger the system becomes, the more likely it is to have poor reproducibility and to become difficult to operate, and the points of concern are cost and effort. Furthermore, since the interpretation of the large volume of data obtained is complicated, advanced scientific and statistical expert knowledge is required. Therefore, construction of test systems and data analysis that are suitable to the purposes of the assessment are most important, and since the US EPA mesocosm study guidelines[29), 30)] on the 0.1 hectare scale, there have been discussions citing various points to be heeded in expert workshops and guidance.[8), 16), 19), 31)–35)] Furthermore, there are large number of presentations at international conferences and in academic papers each year, and the discussion of test systems and assessment methods suitable for refined assessments continues.

With regard to Sumitomo Chemical's pesticides, it is necessary to be concerned about the ecological effects on various species, and with the agents we will introduce in the following, we have been able to demonstrate the safety of these chemicals through microcosm and mesocosm studies, which are higher tier studies, using the most advanced assessment tests and analysis technology.

2. Specific examples

(1) Esfenvalerate

An outdoor multi-species mesocosm can be cited as a test method that integrates species, climate, and the regional characteristics of use. Esfenvalerate, which is a pyrethroid insecticide, has a broad range of sensitivity to aquatic organisms because of its mode of action, and since its impact is great (**Table 6**), and a mesocosm study was conducted in the past in the United States.[36)–38)]

On the other hand, it was thought that an outdoor mesocosm study at an EU site was necessary for assuring safety in the ecosystems within the EU region. In addition, as is found in various types of guidance,[33), 35)] it was thought that there was a danger of producing large changes and destruction of the ecosystem in the test system because the mobility and predation pressure of the fish on other aquatic organisms (invertebrates) would be overwhelming, and assessments should be carried out with separate test systems for fish and invertebrates that were the targets of the assessment. Therefore, it was determined that two test systems would be suitable, an invertebrate mesocosm study evaluating many species and a fish mesocosm study evaluating the short-term and long-term effects on fish and the effects on fish migration from unexposed areas.

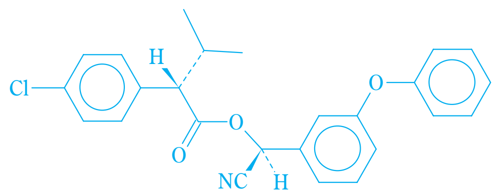
In the two mesocosm studies that were carried out, the effects on diverse invertebrates, the interactions among organisms and community structure of the ecosystem as a whole as well as the effects on individuals migrating into the distribution area and the long-term effects taking into consideration the food chain for fish were evaluated in detail. It was possible to verify the safety of esfenvalerate in the concentrations found in the environment based on actual applications in all cases. These two studies will be introduced in the following.

1) Invertebrate mesocosm study

The mesocosm test system was created by placing stainless steel cylinders known as enclosures, each forming a test vessel, after introducing the natural water and bottom sediment with the organisms collected from the Bodensee (a lake along the borders of Germany, Austria and Switzerland) into a large pool called a test basin, passing through the acclimatization period

Table 6 Brief summary of environmental fate and ecotoxicological profiles of esfenvalerate

Structure :



Core Aquatic Fate Profile :			
Water-Sediment		Dissipation from water phase	33% immediately after application
Core Effect Profile:			
Acute/Short-term			
	Fish	Rainbow trout	96h-LC_{50} = 0.1 – 0.302 µg/L
		Bluegill sunfish	96h-LC_{50} = 0.205 µg/L
		Fathead minnow	96h-LC_{50} = 0.18 µg/L
	Invertebrate	*Daphnia magna*	48h-EC_{50} = 0.228 – 0.9 µg/L
	Aquatic plant	Green alga	96h-EC_{50} = 6.5 – 9.1 µg/L
Chronic/long-term			
	Fish	Rainbow trout	21d-NOEC = 0.001 µg/L
		Fathead minnow*	260d-NOEC = 0.09 µg/L
	Invertebrate	*Daphnia magna*	21d-NOEC = 0.0018 µg/L
		Chironomus riparius	28d-NOEC = 0.16 µg/L

* : Data of fenvalerate

over several months with stabilization and establishment of the ecosystem (**Fig. 4** and **5**).

Fig. 4 Photograph of the test system (invertebrate mesocosm)

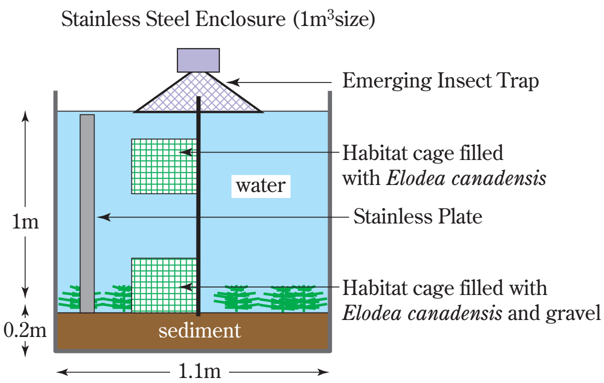


Fig. 5 Schematic view of the enclosure (invertebrate mesocosm)

The test period was set within several months from the beginning of summer to fall when the numbers of individuals for the various species continued in large numbers with stability. To assess the various organisms with differing life histories, habitat and behavior, cages containing gravel and aquatic plants, a stainless steel plate for attached organisms such as snails and traps for catching emerging aquatic insects were placed in each of the enclosures, and the assessments were conducted on a variety of organisms through periodic sampling. Furthermore, for species for which the assessment might be insufficient because of the scarcity of individuals, either mesh cages housing these organisms were introduced separately, or laboratory effect evaluation tests were conducted with exposed water collected from the test system. Not only were the direct effects of chemical treatment evaluated for approximately 80 species including a separation of larva and adult insects, but also the increases and decreases in the numbers of individuals caused by indirect effects including recovery from those effects. Moreover, by using esfenvalerate labeled with the radioactive isotope ^{14}C in the tests, verification of the treatment concentration and detailed assessments of the environmental behavior of the degradation products were carried out through periodic analysis of the water and the bottom sediment. **Fig. 6** shows the behavior of esfenvalerate and its main metabolite 3-phenoxybenzoic acid (PB acid).

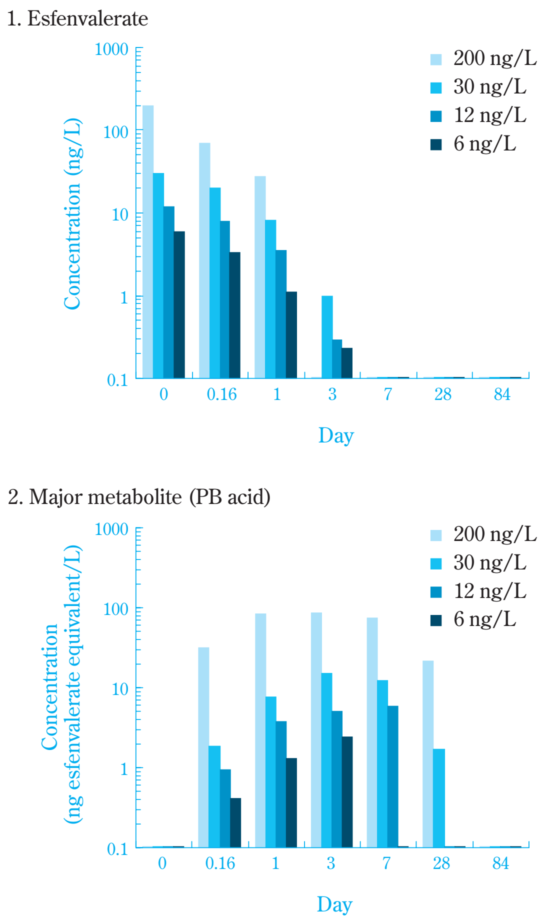


Fig. 6 Concentrations of esfenvalerate and its metabolite in water

The effects on *Alonella* spp. are shown in **Fig. 7** as an example of a biological assessment. To make refined assessments of the ecological effects derived from the substances being tested with the comparatively large variations in the reactions of the organisms in complex test systems, it has been effective to clarify the dose-response reaction parameters, and establishing unrealistically high concentration group as a positive control is useful. As can be seen in Fig. 7, a significant ecologi-

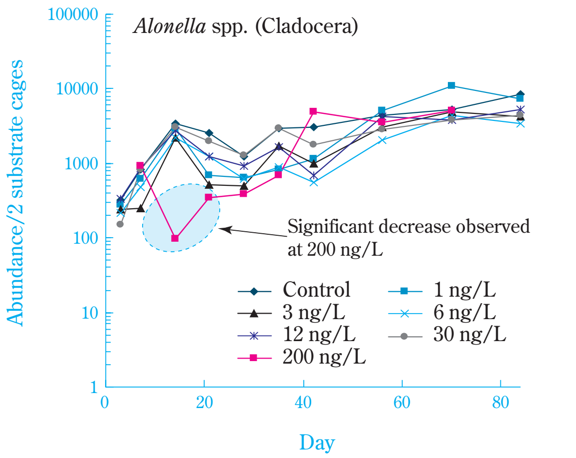


Fig. 7 Graph of the changes in abundance of *Alonella* spp.

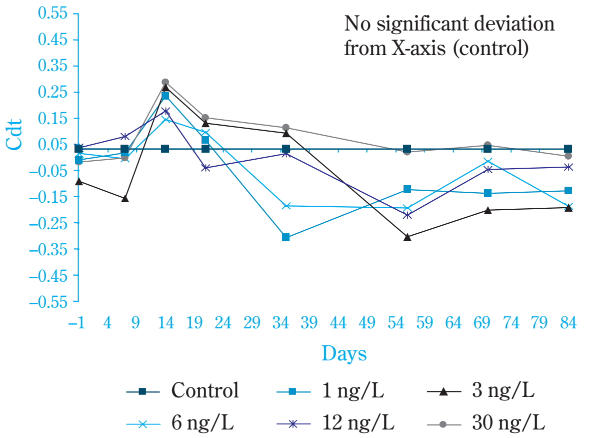


Fig. 8 A typical PRC diagram for community structure evaluation (substrate associated organisms)

cal effect was found during initial distribution in the positive control group (200 ng/L), but that effect was transient, with recovery in approximately 2 weeks.

In addition to these assessments of various organisms, community structure analysis, which is known as the principal response curve (PRC) in a multivariate analysis, is important because analysis of the variety and community structures are important in assessing ecosystems.[22] **Fig. 8** is the organism community PRC (omitting the positive control group) where the inside of the cages (aquatic plants and gravel) is the habitat. Since the X-axis in the graph shows the community structure of the control group, separation shows the differences in community structure between the treated and the control groups.

Accordingly, it can be seen that the separation between all of the concentration groups and the control group throughout the test period was small and there was no effect on the community structure.

Table 7 summarizes the assessment of the ecology in the mesocosm test based on these individual detailed analytical results. Based on guidance[22] from the EU, assessments of the level of the effects on the population and the community were made in five classifications from "no effect" to "irreversible long-term effect."

As a result, the no-observed-effect concentration for the population (NOEC population) was assessed at 6 ng/L and the no-observed-effect concentration for the community (NOEC community) at 30 ng/L. In the end, the no-observed-ecologically-adverse-effect concentra-

Table 7 Summary of the effect classification

	Effect Classification*					
	1 ng/L	3 ng/L	6 ng/L	12 ng/L	30 ng/L	200 ng/L
Population (number of evaluated taxa)						
Phytoplankton (14)	1	1	1	1	1 NOEC	1 – 2
Periphyton (2)	1	1	1	1	1	1 NOEC
Macrophytes (1)	1	1	1	1	1	1 NOEC
Zooplankton (29)	1	1	1 NOEC	1 – 2	1 – 3	1 – 5
Macroinvertebrates (34)	1	1	1	1 NOEC	1 – 3	1 – 5
Community						
Phytoplankton	1	1	1	1	1 NOEC	NA
Open water invertebrate	1	1	1	1	1 NOEC	NA
Substrate associated	1	1	1	1	1 NOEC	NA
Sediment dweller	1	1	1	1	1 NOEC	NA
Emergent insect	1	1	1	1	1 NOEC	NA
Taxonomic Richness	1	1	1	1	1 NOEC	1 – 3
Ecological evaluation including recovery						
NOEAEC					X	

* : Effect classification was based on the EU guidance and summarized as follows :
Class 1 : no effect ; Class 2 : slight effect ; Class 3 : short term effect ; Class 4 : long term effect with recovery ; Class 5 : irreversible long-term effect

tion (NOEAEC), which takes recovery into consideration, was assessed at 30 ng/L.[39]

2) Fish mesocosm study

The test system can be set up in a similar manner to the test system for invertebrates, but based on the various considerations, such as the level of fish predation pressure, the size of the biomass, maintaining the quality and other organisms and methods of observation, a larger test system was used, housing cages set up and the design made for contact between the bottom sediment and fish in long-term exposure (**Fig. 9** and **10**).

Fig. 9 Photograph of the test system (fish mesocosm)

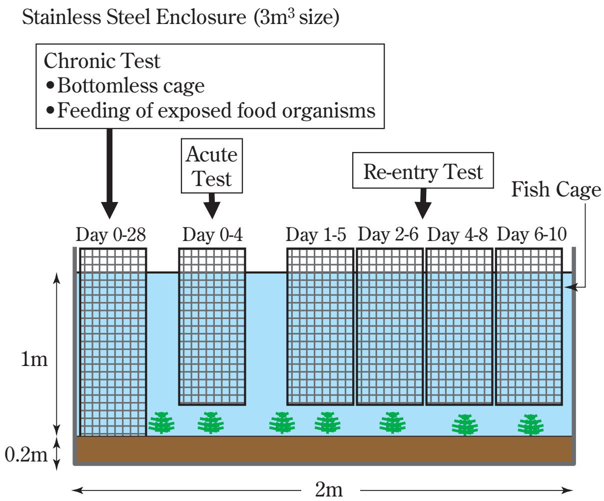
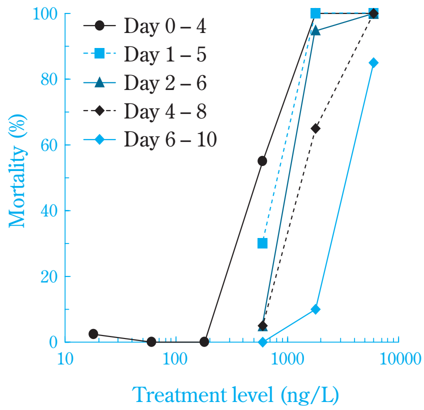


Fig. 10 Schematic view of the enclosure (fish mesocosm)

Assessment of acute ecological effects was carried out by exposing fish separated out in cages to the water system. On the other hand, assessment of long-term effects was carried out with the cage bottoms removed envisioning contact (feeding activity) of the fish with the bottom where the substance being tested was adsorbed. Furthermore, the supplemental feeding was carried out using food organisms (benthic worms and midges, prepared in multiple small containers) that had been exposed to esfenvalerate separately, and an assessment test system closer to reality was constructed by considering exposure throughout the food chain. Furthermore, to examine the effects on fish migrating from unexposed areas, cages containing the fish were added after treatment with the substance being tested, and mitigation of the ecological effects were verified. As is shown in **Fig. 11**, the acute LC_{50} value for rainbow trout in the mesocosm test system was approximately 5 times higher than the results from the lower tier tests at 550 ng/L, and the effects on the fish that were added after distribution clearly dropped all with the passage of time. It was possible to demonstrate the capacity for recovery from the ecological effects for the populations including migrating fish.

In addition, in the tests assessing the long-term func-



	96h-LC_{50} (ng/L)
Day 0 – 4	550
Day 1 – 5	770
Day 2 – 6	1040
Day 4 – 8	1450
Day 6 – 10	3510

Fig. 11 Results of the acute and re-entry tests

Table 8 Results of the chronic rainbow trout test

	Survival (%)	Growth rates (r)	
		Wet weight	Total length
Control	100	1.5 ± 0.026	0.25 ± 0.010
18 ng/L	95	1.4 ± 0.054	0.25 ± 0.019
60 ng/L	100	1.5 ± 0.004	0.23 ± 0.024
180 ng/L	100	1.4 ± 0.052	0.23 ± 0.011
NOEC	180 ng/L	180 ng/L	180 ng/L
Overall NOEC		180 ng/L	

$r = 100 \times (\log A_{28} - \log A_0) \times (t_{28} - t_0)^{-1}$

A_{28} : weight or length of Day 28

A_0 : weight or length of Day 0 in fish culture

t_{28}, t_0 : time (days) of test initiation and termination, i.e. Day 0 and Day 28

(OECD guideline 215)

tional effects through the food chain because of the feeding of organisms exposed to esfenvalerate, the effects on growth, of which rates of increase in body weight and body length are representative, were evaluated precisely. It was possible to verify the safety of esfenvalerate for fish at a maximum test concentration of 180 ng/L, which is much higher than the predicted concentration in the environment and would not happen in reality (**Table 8**).[39]

(2) Pyriproxyfen

Pyriproxyfen, which is an insect growth regulator and classified as a juvenile hormone analog for insects, is thought to impose limited effects on organisms in the environment due to its mode of action. In fact, the sensitivity of fish and algae is low as shown in **Table 9**, and while only cladoceran (i.e. water fleas) show a significantly reduced reproduction among invertebrates at low concentrations, it was found that other organisms such as asellus, copepods, midges, and shrimp exhibited a weak ecological effect of one order or more.

To examine the ecological effects on cladoceran in detail, the environmental fate of pyriproxyfen was studied in a water-sediment system simulating the natural hydrosphere. The decay of pyriproxyfen in water is rapid, and the effects on the reproduction of cladoceran rapidly disappear when they are transferred to clean water (Table 9).[40] Therefore, the effects of pyriproxyfen on cladoceran either do not appear in the natural environment or even if we assume that they do appear, are transient. The changes in the community in the ecosystem, including competitors, can be thought of as being within an acceptable range.

To examine the ecological effects on cladoceran in greater detail, a laboratory plankton community microcosm study was conducted as a higher tier test system. Natural water and sediment (The Netherlands) including plankton communities were introduced into 20 L test vessels in the laboratory (**Fig. 12**).

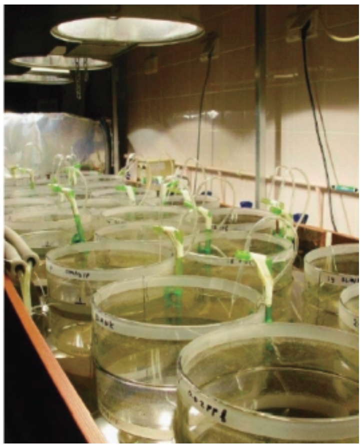
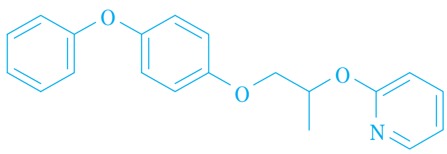


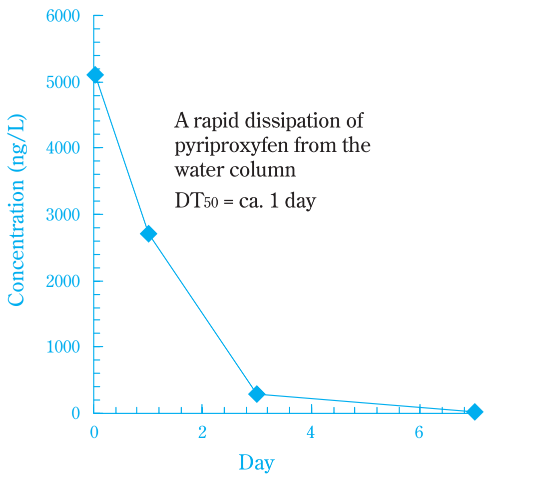
Fig. 12 Test system of the indoor multi-species zooplankton dominated microcosm

Table 9 Brief summary of environmental fate and ecotoxicological profiles of pyriproxyfen

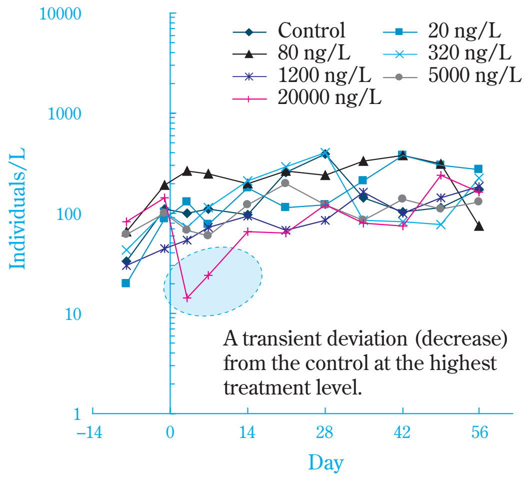
Structure :			
Core Aquatic Fate Profile :			
Water-Sediment		DT50 in water phase	1.4 – 1.5 days
Core Effect Profile :			
Acute/Short-term			
	Fish	Rainbow trout	96h-LC50 = 218 – > 325 μg/L
		Bluegill sunfish	96h-LC50 > 270 μg/L
	Invertebrate	*Daphnia magna*	48h-EC50 = 187 – 400 μg/L
	Aquatic plant	Green alga	72h-EC50 = 64 – 66 μg/L
Chronic/long-term			
	Fish	Rainbow trout	95d-NOEC = 4.3 μg/L
	Invertebrate	*Daphnia magna*	21d-NOEC = 0.015 μg/L
		Chironomus riparius	28d-NOEC = 10 μg/L
		Asellus hilgendorfii	19d-NOEC = 10 μg/L
		Tigriopus japonicus	8d-NOEC = 10 μg/L
		Mysidopsis bahia	28d-NOEC = 0.81 μg/L
		Daphnia pulex	Recovered after 1.88 μg/L exposure
		Recovery in clean water	

Since this was a comparatively small test system in a laboratory, there were some limitations in degradation of test substance (sunlight photolysis etc.), and adaptability of large organisms and ones capable of flight, but it is known to be capable of refined assessments of plankton community reactions in particular.[22] As is shown in **Fig. 13**-1, the pyriproxyfen concentration rapidly decreased after treatment, and it was confirmed that evaluation of ecological effects, including the environmental fate of pyriproxyfen was possible with this test system. The reduction in the population of cladoceran was found only for a short time in the unrealistic maximum concentration group, and that population rapidly recovered to the level of the untreated group (Fig. 13-2). A transient increase (indirect effect) in the population of rotifers, which compete with cladoceran for food, was found (Fig. 13-3), but as is shown in the PRC graph (Fig. 13-4), the change in the community structure of the plankton as a whole was transient, and in the latter half of the test, the separation from the X-axis, which is the deviation from the untreated group, disappeared, and it was possible to show a recovery in the community structure. The NOEAEC, which takes into consideration the recovery in this test, showed that this was a maximum test concentration group with an unrealistically high concentration.

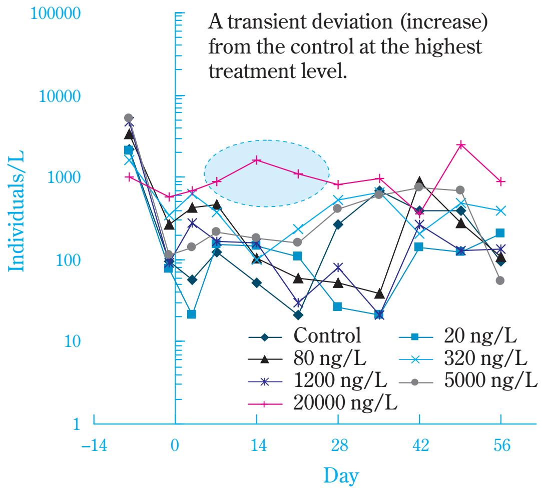
Moreover, a characteristic of cladoceran is their breeding system, and they have a unique reproductive strategy where, normally, offspring are born and propagated through parthenogenic reproduction in female individuals only (Table 1, Parthenogenic reproduction in arthropoda), but with changes in environmental con-



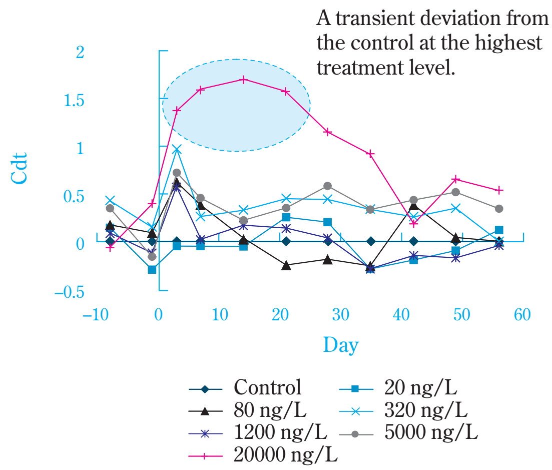
1. Dissipation of pyriproxyfen from water column of microcosm test system (5000 ng/L treatment)



2. Population changes of Cladoceran in the microcosm



3. Population changes of Rotatoria in the microcosm



4. Principle response curve (PRC) analysis of zooplankton community in the microcosm

Fig. 13 Summary results of the indoor multi-species microcosm study of pyriproxyfen ditions such as the season, lower temperatures, short days and insufficient food, male individuals are born and eggs that can last over the winter known as resting eggs are laid through bisexual reproduction. The effect of juvenile hormone analogues for insects on the specific reproduction of cladoceran as a whole has undergone a variety of research in recent years, and it has become clear that the offspring born in high concentrations are normally not only female individuals but also an increased proportion of male individuals.[41)–43)] However, it was found that the birth of male individuals because of exposure to pyriproxyfen is clearly not only with high concentration exposure exhibiting reduced reproduction, but also has a rapid disappearance of the changes in the same manner as the other effects on reproduction when cladoceran is transferred to clean water, and there is a clear recovery to only female offspring being born. In addition, the male individuals that are born have been confirmed to have healthy growth and actively release sperm, so they exhibit reproductive functions.[44)] Because of this, it was verified that the significance of the environmental effect evaluation on the reproduction of male individuals in high concentration exposure to pyriproxyfen is extremely low.

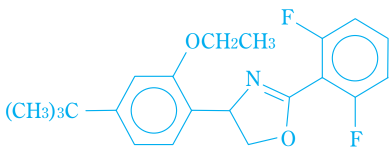
(3) Etoxazole

The variations due to differences in sensitivity between the standard species used in tests for assessing ecological effects and the wild species as well as the differences in environmental conditions are some of the most important factors in refined ecological assessments. This uncertainty factor is considered in lower tier TER, uncertainty coefficients and standard RQ values by each authority, but for example, the TER standard of 100 for acute ecological effects in the EU, that is 100 times the ratio of the exposure, is sometimes an extremely strict standard for assessments of sensitive species, and higher tier assessments are necessary. In terms of higher tier tests for refined evaluations in such cases, local varieties and refined evaluations under environmental conditions of biodiversity and suitable areas are necessary. Although outdoor test systems introduced as above for esfenvalerate can be supposed, there is a possible difficulty arising in refined evaluations using these test methods because of various limitations, such as suitable test facilities, securing test equipment, predicting weather conditions, and control of the ecosystem of the test system. In some cases, test systems may be ruined by floods and the tests themselves become impossible. When there are limits to the scope of concern for organisms and effects, setting up special site conditions in laboratory test systems constructed of water, bottom sediment and species from the site can be considered, and an indoor microcosm test was implemented to make a refined assessment of Southern Europe for verifying the safety of etoxazole.

The effects of exposure to etoxazole, which is a miticide with molting inhibitor activity, were comparatively

Table 10 Brief summary of environmental fate and ecotoxicological profiles of etoxazole

Structure :



Core Aquatic Fate Profile :			
Water-Sediment		DT_{50} in water phase	0.4 – 3.0 days
Core Effect Profile :			
Acute/Short-term			
	Fish	Rainbow trout	96h-LC_{50} = 2800 μg/L
		Bluegill sunfish	96h-LC_{50} = 1400 μg/L
	Invertebrate	*Daphnia magna*	48h-EC_{50} = 2.0 – 7.1 μg/L
		Chironomus riparius	10d-LC_{50} > 56000 μ/kg
	Aquatic plant	Green alga	72h-EC_{50} > 10000 μg/L
Chronic/long-term			
	Fish	Rainbow trout	89d-NOEC = 15 μg/L
	Invertebrate	*Daphnia magna*	21d-NOEC = 0.2 μg/L
		Chironomus riparius	10d-NOEC = 25000 μg/kg

strong for cladoceran, which are an arthropod like mites with their comparatively short molting cycle (under laboratory room temperature: approximately one to two day intervals for young offspring and approximately three day intervals for adults) not only in its long-term ecological effects but also in its acute effects, but it was understood from its mode of action that the effects on many other aquatic organisms would be small (**Table 10**).

While etoxazole rapidly disappears from the water column in a water-sediment system, it was supposed that a detailed assessment of the effects on plankton communities including various species of cladoceran during initial exposure was necessary for a refined evaluation. In addition, the temperature, length of the days, in rich nutrient state causing a high algal growth, etc., in the Southern European summer had to be considered, but setting the various conditions was achievable with comparative ease through temperature and light control in the laboratory and adjusting the addition of nutrient salts. The aquatic ecosystem in an agricultural region of the site (Spain) was surveyed and collected, and in microcosm test system with a size of 100 L was constructed in a laboratory from the water, bottom sediment and aquatic plants, which are the main components. A test system that incorporated a diverse plankton community was constructed by setting up a cage housing aquatic plants which are an important habitat for various zooplankton. As is shown in **Table 11**, a detailed assessment of a total of 12 classes of zooplankton such as rotifers, Copepoda, Cladocera and Ostracoda was possible using the population levels in this test system. With the effect of a reduction in the number of individuals, even though it was short-term, *Simocephalus vetulus*, which is one species of cladoceran, was found to be a sensitive species in the maximum concentration group. In addition, there was a short-term increase in rotifers, which are a community of organisms that competes with them for food. However, no effect was found on the total number of plankton individuals, the diversity of the organisms or the community structure even in the maximum concentration group. The NOEAEC value in this test was determined to be the maximum concentration tested, and the acceptability near an acute EC_{50} value for the most sensitive species in standard tests for the organism communities at the site was demonstrated.

Table 11 Summary results of the indoor multi-species microcosm study of etoxazole

		Effect Classification*		
		0.2 µg/L	0.66 µg/L	1.54 µg/L
Population				
Phytoplankton (Chlorophyll-a)		1	1	1 NOEC
Total Rotatoria		1	1 NOEC	2(increase)
Copepoda	Copepoda	1	1	1 NOEC
	Cyclopoida	1	1	1 NOEC
	Nauplia	1	1	1 NOEC
Cladocera	*Simocephalus vetulus*	1	1 NOEC	2(decrease)
	Chydoridae spp.	1	1	1 NOEC
	Chydorus spp.	1	1	1 NOEC
	Alona spp.	1	1	1 NOEC
	Alonella spp.	1	1	1 NOEC
	Graptoleberis testudinaria	1	1	1 NOEC
	Pleuroxus spp.	1	1	1 NOEC
Total Ostracoda		1	1	1 NOEC
Total Crustacea		1	1	1 NOEC
Total zooplankton		1	1	1 NOEC
NOEC population			X	
Community				
Taxonomic Richness		1	1	1 NOEC
Community Structure		1	1	1 NOEC
NOEC community				X
NOEAEC				X

* : Effect classification was based on the EU guidance and summarized as follows :
Class 1 : no effect ; Class 2 : slight effect ;
Class 3 : short term effect ;
Class 4 : long term effect with recovery ;
Class 5 : irreversible long-term effect

Future Outlook

Because of the complexity of the natural environment, organisms and ecosystems, a large number of points that have yet to be clarified and the difficulty of making assessments related to these, current assessment systems, assessment methods, analytical methods experimental methods, etc., leave problematic points as well as obscure and unexplained points. Industry, academia and government are continuing to work on improvements and proposals for more research and investigations into methods for assessing environmental impact in various areas. The OECD test guidelines for assessing ecological impact have reached 25 methods (March 2008), and investigations are presently being made into new species and test methods for standard laboratory tests for lower tier evaluations. In addition, further development and increased practicality in the future is desirable in the test and assessment methods for endocrine disruptor compounds that are under various investigations in various areas starting with the OECD, and the various methods proposed in AEDG,[16] HARAP,[8] ECOFRAM,[19] EUPRA,[45] the "Registration Withholding Standards of Agricultural Chemicals concerning Prevention of Damage to Aquatic Animals and Plants" report from the Ministry of the Environment Government of Japan,[11] the FOCUS Working Group report[24] and various other reports, etc., for which use is still insufficient, that is probabilistic methods, advanced region specific simulations, meta-population modeling, test methods for simulating flow-through microcosm, etc. In addition, there are expectations for further investigations in the future into the use of various QSAR toxicity prediction methods, genomics techniques that are used in pharmaceutical development and others that will be useful in the future. We are continuing discussions, applications and collection of knowledge about the microcosm and mesocosm test techniques introduced here, and moving forward, progress toward greater know-how, and refined assessments by building up technology are desirable. We would like to confirm the safety of Sumitomo Chemical's pesticides in the ecosystems and carry out development of pesticides that are more environmentally benign by driving these new technologies forward.

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