Ecotoxicological Risk Assessment of Pesticides in Terrestrial Ecosystems

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Ecotoxicological risk assessment of pesticides in terrestrial ecosystems in particular has become one of the most important areas in scientific pesticide evaluation for consideration of biodiversity preservation. In order to develop safer pesticides, Sumitomo Chemical Co., Ltd. has been evaluating our pesticides by utilizing scientific knowledge and state-of-the-art techniques in accordance with the latest regulatory criteria for the ecological risk assessment in complicated terrestrial ecosystems. In this article, outlines of the terrestrial ecological risk assessment procedures in the EU, USA and Japan are comparatively summarized and some examples of higher-tier ecotoxicological studies undertaken to demonstrate that our pesticides are benign to the terrestrial environment are introduced along with the recent progress in our research.

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Introduction

With the increasing awareness of environmental protection worldwide, it is becoming more and more important to evaluate the effects of chemical substances on the natural environment. With pesticides being intentionally released into the natural environment to protect crops from harmful insects, weeds and pathogenic microorganisms, countries worldwide, with Europe and the United States taking the lead, have formulated regulatory procedures making environmental risk assessments indispensable registration requirements.[1), 2)] Among them, the assessment of ecological risk is utilized to formulate the policies and measures suitable for protecting the ecosystem by understanding the impact of chemical substances prior to their emission into the environment. The most important issue is accurate assessment of the risks to the organisms in the environment that make up the ecosystem.

The assessment of the ecological impacts of pesticides is classified into assessments for aquatic ecosystems and assessments for terrestrial ecosystems in the area of pesticide application followed by migration. Methods for assessing the risks to aquatic ecosystems have been debated worldwide ahead of those for terrestrial ones because, in addition to its being comparatively easy to consider the single hierarchical structure from producers such as algae to multiple levels of consumers such as crustaceans and fish, these aquatic plants and animals are food.

Terrestrial ecosystems, on the other hand, provide an opportunity (blessing) for all species, including humans, to survive, just like the aquatic ecosystems, and the assessment of pesticide impact therein becomes one of the most important issues in the safety assessment. However, evaluation of the risk to terrestrial ecosystems, which maintain interrelationships such as predation, prey, competition and parasitism among the wide variety of living species in a complex mixture of the environmental media of air, soil and water systems, is extremely high-level and complex when compared with evaluation of aquatic ecosystems. In addition, the mixing of a wide variety of ecosystems with unique structures and functions due to the everyday activities of people and the artificial connections with pesticides and agricultural activities is a factor that brings about further complexity as in the case of apiculture. Furthermore, there are many species of organisms for which the ecology is unknown, and the assessment of terrestrial ecological risks becomes more difficult because it is coupled with a paucity of toxicological knowledge on terrestrial organisms.

To develop pesticides that may be used with more confidence in their safety, Sumitomo Chemical is con-

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firming their safety by carrying out assessments of the impact on these complex and obscure terrestrial ecosystems based on preserving biodiversity. These assessments are driven by state-of-the-art assessment techniques based on the most recent trends in regulations worldwide. In this article, we outline assessments of terrestrial ecological risks in Europe, the USA and Japan, give some recent specific examples that demonstrate the safety of our pesticides for organisms in the environment using special higher tier evaluation systems designed for more exhaustive assessments, and introduce some recent investigations on the issues to be resolved in the future.

Assessment of Terrestrial Ecological Risk

1. Concepts for assessing terrestrial ecological risk

After outlining the diversity and characteristics of the ecosystems, that is, the natural environment and organisms in the environment, that are the target of assessments of ecological risks, we will give a simple introduction to basic terrestrial risk assessment techniques.

(1) Natural environment and organisms in the environment

According to the Convention on Biological Diversity,[3] an ecosystem is a "dynamic complex of plant, animal and micro-organism communities and their non-living environment interacting as a functional unit." With terrestrial ecosystems, the organism part is divided variously according to the functions performed in the ecosystem, and there are three of these divisions: producers generally made up of green plants; herterotrophic organisms that are consumers; and decomposers (microorganisms, etc.) that feed on and mineralize excretions and corpses of both. The consumers may also be divided according to their order in the food chain into primary consumers (herbivores), secondary consumers (carnivores) as well as tertiary and quaternary consumers. However, unlike aquatic ecosystems with their easily conceived interrelationships in a hierarchical structure and the food chain for the ecosystem, the organisms in terrestrial ecosystems have a variety of means of mobility and feeding habits. In addition, since there are also species that change feeding habits according to environmental conditions, the structure of the food chain forms a complicated food web rather than being simple.[4]

On the other hand, approximately 1.75 million known species and 30 million species if the unknown ones are included currently inhabit the Earth,[5] and of these, the total number of terrestrial species is estimated to be approximately 10 million.[6] There are already, for example, 4500 species of mammals, 8650 species of birds, 5000 species of reptiles and 2000 species of amphibians known among the known vertebrates,[7] but there are many species of organisms for which the life cycles, lifestyles, behavioral patterns, types of feed and rates of intake as well as reproductive strategies are unknown.

(2) Basic methods for assessing terrestrial ecological impact

A list of the targeted species and required test data for assessments[8)–13)] in Europe, the USA and Japan are given in **Table 1**. The differences in habitat, forms of agriculture, weather and climate, and the culture, ideas, values, industry and other aspects of human society are reflected here, and there are a variety of differences. However, this covers a wide range of groups in the classification of organisms, including the plants as a basis of the terrestrial ecosystem, invertebrates that play roles such as soil organisms, food organisms, pollinating organisms and natural enemies as well as vertebrates including the birds and mammals that are positioned at the top of the phylogenetic classification.

On the other hand, the basic concepts of assessment methods are common to both Europe and the United States, which early on introduced assessments of the risks of pesticides to terrestrial ecosystems, which are both complex and obscure. While there are some hazard-based assessments, they are basically risk-based methods that compare and evaluate toxicity and estimated exposure concentrations in various targeted organisms.

Risk-based assessments become increasingly more precise in stages moving toward the natural environment in terms of toxicity and exposure as shown in **Fig. 1**, that is, they progress in a tiered structure. The toxicity values obtained during a test window, for example, in initial lower tier assessments by standardized tests prescribed by the Organization for Economic Cooperation and Development (OECD) or the US Environmental Protection Agency (EPA) and the ratio (toxicity/predicted concentration in the environment or predicted exposure concentration) with predicted concentration in the environment and predicted exposure concentration under the worst-case conditions foreseen

Table 1 Data requirements in pesticide registration for representative outdoor use patterns in EU, US and Japan

Taxonomic groups			Required data	EU	US	JP
Vertebrates	Birds	Tier-1	Acute: Acute oral study	R	R	R
			Short term: 5-day dietary study	CR	R	CR
			Long term: Reproduction study	CR	R	NR
		Higher tier	Avoidance/Palatability test for bait, granules and treated seeds	CR	NR	NR
			Pen/Cage test	CR	NR	NR
			Wild birds: Field test	CR	CR	NR
	Mammals	Tier-1	Acute: Acute oral study	R	R	NR
			Long term: Reproduction study (or Teratology study)	R	R	NR
		Higher tier	Wild mammals: Acute study	NR	CR	NR
			Pen/Cage test	CR	NR	NR
			Wild mammals: Field test	CR	CR	NR
Invertebrates	Bees	Tier-1	Acute oral study	R	NR	R*
			Acute contact study	R	R	
			Bee brood feeding study	CR	NR	NR
		Higher tier	Residue test	CR	CR	NR
			Cage/Tunnel test	CR	NR	NR
			Field test	CR	CR	CR
	Other arthropods	Tier-1	Standard laboratory study	R	NR	R
		Higher tier	Extended laboratory/Aged residue test	CR	NR	NR
			Field test	CR	NR	CR
	Silkworm	Tier-1	Acute: Acute oral study	NR	NR	R
		Higher tier	Residue test	NR	NR	CR
Soil organisms	Earthworms	Tier-1	Acute: Acute study	R	NR	NR
			Chronic: Reproduction study	CR	NR	NR
		Higher tier	Field test	CR	NR	NR
	Soil non-target micro-organisms	Tier-1	Soil nitrification and carbon mineralisation study	R	NR	NR
		Higher tier	Field test	CR	NR	NR
	Other soil non-target macro-organisms	Tier-1	Collembola/Gamasid mite: Reproduction study	CR	NR	NR
		Higher tier	Litter bag test	CR	NR	NR
Plants	Non-target plants	Tier-1	Screening data such as phyto-toxicity, Seedling emergence/Vegetative vigor study	R	R	NR
		Higher tier	Field test	CR	CR	NR

R: Required, CR: Conditionally Required, NR: Not Required
*: either of the acute oral or acute contact studies

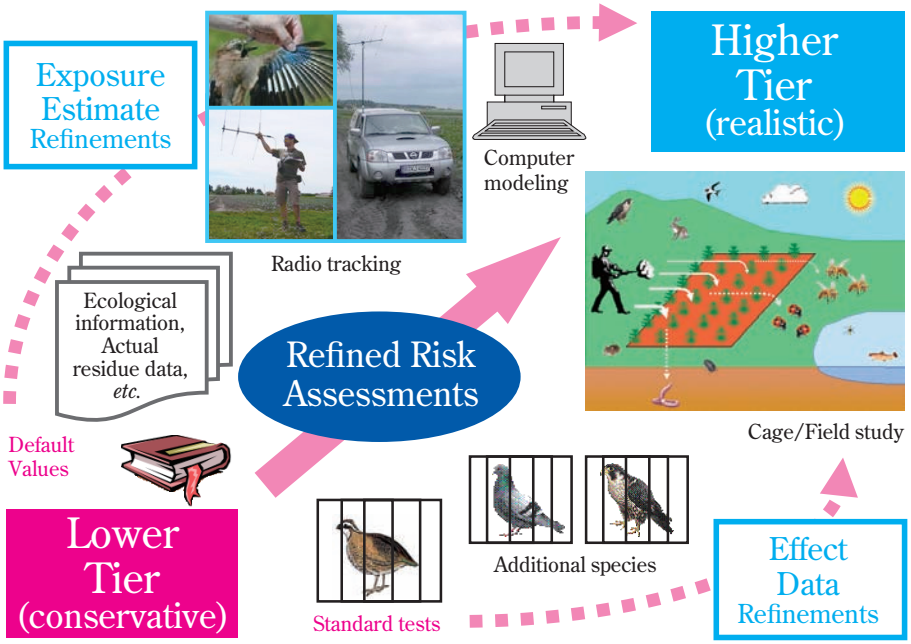


Fig. 1 General principle of the ecotoxicological risk assessment scheme in the terrestrial ecosystems

if the greatest amount of pesticide migrates to the area in question are found, and assessments are made by comparisons with conservative allowance standards determined by the regulatory authority. When safety is not sufficient with this method, higher tier risk assessments, which are closer to reality, are carried out.

In higher tier risk assessments, the precision of predicted concentrations in the environment is normally increased using various indices that include distribution and degradation of pesticides from the standpoint of reassessing the exposure assessments under the worst conditions, which were used in the lower tier assessments. In addition, there are also methods that incorporate increased precision in predicted exposure concentrations using information on behavioral patterns such as habitat, feeding habits and breeding of the organisms being studied or concentrations of pesticides in food under actual conditions. On the other hand, various methods such as incorporating evaluations of safety that acquire toxicity values through higher tier tests under test conditions closer to the actual environment are implemented from the standpoint of reassessing the toxicity indices in laboratory tests under the standardized test conditions used in lower tier assessment. There is no uniform test planning for these higher tier assessments, and this means special planning that takes in consideration the points of concern and extent for the species being evaluated and the ecological impact.

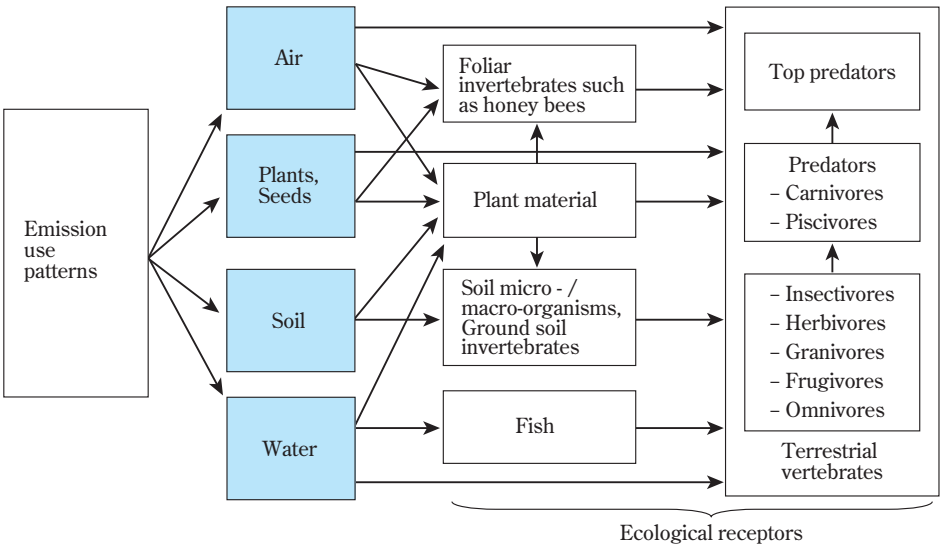
2. Assessment of terrestrial ecological risk in various regions (EU, USA and Japan)

Specialization has been found for each region and each group of organisms in the assessment of ecological risks for registering pesticides. In the following, we will introduce methods for the assessment of terrestrial ecological risks in Europe, the USA and Japan.

(1) EU

It can be said that the EU is the region with the most advanced preparations for a system of terrestrial ecological assessment methods. **Fig. 2** is a conceptual diagram[14] showing assessments for terrestrial ecosystems that form the general basis for pesticide registration in the EU. The interrelationships between the environmental media (compartments) and organisms in the environment (ecological receptors) and the concepts for considering the environmental dynamics of pesticides are shown, and the food chain is taken into account.

The required data and the indices[8)–10)] used in assessments are given in **Table 2**. Next, we will give an overview of assessment methods for various groups of organisms using various indices in order of precedence. Moreover, in the EU, organisms living in agricultural lands such as soil organisms and honey bees, beneficial arthropods and other agricultural materials are also evaluated, and methods for increasing precision in the exposure level from the lower tier stages are shown. They are characterized by giving flexibility to risk assessment methods.



Chemicals reach the initial exposed compartment (s) (Air, Plants, Seeds, Soil or Water), distribute in the environment and reach ecological receptors.

Fig. 2 EU Conceptual model for the hazard and risk assessments in the terrestrial ecosystems

Table 2 Data requirements and parameters for exposure/risk for the pesticide registration in EU (Tier-1)

Taxonomic groups	Required data	Endpoints	Parameters for exposure	Parameters for risk	Acceptable trigger of TER or HQ
Birds	Acute: Acute oral study	LD_{50}	DDD	TER	≥ 10
	Short term: 5-day dietary study	LC_{50}			≥ 10
	Long term: Reproduction study	NOEC			≥ 5
Mammals	Acute: Acute oral study	LD_{50}	DDD	TER	≥ 10
	Long term: Reproduction study (or Teratology study)	NOEL			≥ 5
Bees	Acute oral and contact study	LD_{50}	Application rate	HQ	< 50
	Bee brood feeding study	NOEC	Application concentration	TER	≥ 1
Other arthropods	Standard laboratory study	LR_{50}	Exposure rate	HQ	< 2
Earthworms	Acute: Acute study	LC_{50}	PEC soil	TER	≥ 10
	Chronic: Reproduction study	NOEC	PEC soil	TER	≥ 5
Soil non-target micro-organisms	Soil nitrification and carbon mineralisation study	NOEC	PEC soil	TER	≥ 1
Other soil non-target macro-organisms	Collembola/Gamasid mite: Reproduction study	NOEC	PEC soil	TER	≥ 5
Non-target plants	Screening data such as phytotoxicity	NOEC	Application rate	TER	≥ 1

DDD: Daily Dietary Dose, TER: Toxicity Exposure Ratio, HQ: Hazard Quotient
PEC soil: Predicted Environmental Concentration in soil

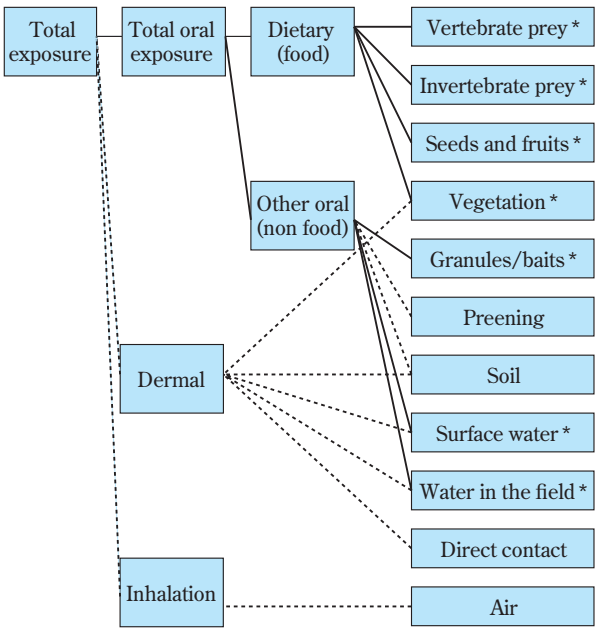
(I) Birds and mammals[8)–10), 15)]

The ratio of the toxicity index (endpoint) for each test and estimated theoretical exposure is calculated as the toxicity exposure ratio (TER, endpoint/estimated theoretical exposure), and safety is assessed by comparison with a standard allowable value for safety (acceptable trigger value).

In lower tier assessments, acute oral test data is required for one species of bird (bobwhite or Japanese quail), and when exposure of parent animals and exposure of a nest site during the breeding period from the pattern of use of pesticides cannot be ruled out, data from avian reproduction tests for one species is necessary. On the other hand, in the evaluation of wild mammals, data from acute oral tests in rats and long-term experiments such as two-generation reproduction tests through human health risk assessment are used.

The endpoints for various tests are 50% of the lethal dose (LD_{50}) in acute oral tests and no observed effect concentration (NOEC) or no observed effect level (NOEL) in reproduction tests. Of these, NOEC, which is expressed as the concentration per unit in food (mg/kg or ppm diet) is converted to NOEL (units being mg/kg b.w./day; the dose per kg body weight based on food consumption) and then used in the assessment.

The concept of the EU exposure routes (**Fig. 3**) is that the oral route, which is thought to have the greatest frequency and amount of exposure, is positioned as the most important route. Oral exposure scenarios for food and drinking water as well as exposure scenarios from the concentration in organisms are considered, and the estimated theoretical exposure is calculated. Incidentally, there are also examples of exposure scenarios according to treatment methods (foliar spray applications, granular application to the soil, seed treatment) in the EU assessment scheme, but here we will introduce foliar application by spraying on leaves and stems, which is a typical treatment method.



* : covered by Tier-1 scenarios

Fig. 3 Relevant exposure routes dependent on the plant protection product to be assessed in EU

For example, daily dietary dose (DDD) is calculated by multiplying food intake rate per body weight (FIR/b.w.) by the predicted concentration in diet after pesticide application (C). Factors such as the avoidance factor (AV), fraction of diet obtained in the treated area (PT) and fraction of food type in diet (PD) are used in calculating exposure, but in lower tier assessments, the default value is set at 1.

DDD = (FIR/b.w.) * C * AV * PT * PD

DDD: Daily Dietary Dose (mg/kg b.w./day)
FIR : Food intake rate of indicator or generic focal species (g fresh weight/day)
b.w. : Body weight (g)
C : Concentration of compound in fresh diet (mg/kg diet)
AV : Avoidance factor (0: no avoidance, 1: complete avoidance)
PT : Fraction of diet obtained in treated area (0~1)
PD : Fraction of food type in diet (0~1)

In addition, exposure through drinking water is assessed according to leaf scenarios and puddle scenarios for estimating exposure from the application solution pooling in the leaf whorls of plants and puddles on soil.

When these assessments are less than the acceptable trigger value for TER, it means further assessments of safety through various refinements are carried out. For example, with dietary exposure, the actual concentrations in food may be measured, and the default values (AV, PD, PT, etc.) are refined based on databases such as BIRD BIBLE[16] and MAMMAL BIBLE[17] where published references in various feeding habits are compiled. Refinement of estimated theoretical exposure by limiting further the targeted species that can actually be exposed by considering the period of application, or analyzing toxicity patterns in existing tests or in tests with additional species, population modeling, carrying out outdoor field tests,[10] etc. may be considered.

Moreover, because of possible concerns about bioconcentration and the food chain, evaluations of birds and mammals that eat earthworms, evaluations of birds and mammals that eat fish and evaluations of biomagnification are carried out when the octanol-water partition coefficient (logPow) for the pesticide is greater than 3. In evaluations of biomagnification, the food chain for terrestrial vertebrates is envisioned, and predators such as falcons and foxes that prey on animals in lower trophic levels as well as top predators such as eagles, lynxes and wolves are assessed.

(Ⅱ) Bees[8), 9), 15)]

Other than for limited use of pesticides where exposure of honey bees cannot be considered, such as food storage in enclosed spaces and use in greenhouses without pollinators, acute oral and acute contact toxicity tests are necessary for honey bees (*Apis mellifera*). The endpoint used for risk assessment is LD_{50} (μg/bee), and the hazard quotient (HQ, application rate (g/ha)/ LD_{50}) is then calculated. There is considered to be low risk if HQ is less than 50. On the other hand, when a chemical compound may act as an insect growth regulator (IGR), bee brood feeding tests, which assess the risk to the honey bee larvae, are necessary. If HQ ≥ 50 in risk assessments, safety is assessed in higher tier tests such as residue tests, field cage tests, tunnel tests and field tests.

(Ⅲ) Non-target arthropods (beneficial insects other than bees)[8), 9), 15), 18)]

As with honey bees, tests are necessary for cases other than for limited use of pesticides where exposure need not be considered. According to the ESCORT 2 guidance document[18] which describes implementation and assessment, selection of species of organisms to be tested according to the intended use pattern of pesticide includes two standard sensitive species (parasitic wasp: *Aphidius rhopalosiphi* and predatory mite: *Typhlodromus pyri*), and tests where the pesticide is applied to a glass plate are carried out and 50% of the lethal rate (LR_{50}), which is the application rate for 50% mortality, is calculated. The in-field and off-field HQ (predicted exposure/LR_{50}) are calculated from LR_{50} obtained and the predicted exposure (g/ha), and when HQ is less than 2, there is considered to be low risk. In predicting the exposure, the in-field exposure is calculated by multiplying the application rate and the multiple application factor (MAF). Off-field exposure is calculated by multiplying the application rate, MAF, drift factor and vegetative distribution factor. Moreover, in HQ calculations, the diversity of arthropods is considered, and in off-field assessments consideration is given at 10 times the species diversity uncertainty factor.

If HQ ≥ 2 in risk assessments, the number of species tested is increased, and risks are assessed using higher tier tests. In higher tier tests, safety is confirmed using tests such as extended laboratory tests where actual

plant leaves are used instead of glass plates, aged residue tests, and outdoor field tests. Incidentally, the criteria for evaluation in higher tier tests focus on recovery from effects, and recovery is prescribed as within 1 year for in-field assessment and within an ecologically allowable period for off-field assessment.

(Ⅳ) Soil organisms[8), 9), 15)]

When soil applications or soil contamination is foreseen, assessments on corresponding species are necessary. Besides earthworms, which are a typical species, soil micro-organisms and in some cases, soil macro-organisms other than earthworms must be assessed.

(i) Earthworms

LC_{50} is derived from acute toxicity tests using OECD artificial soil with earthworms (*Eisenia fetida*) as the test species, and TER (LC_{50}/PEC soil), which is a ratio of the predicted environmental concentration in soil (PEC soil, soil depth 5 cm, soil density 1.5), is calculated. If TER ≥ 10, there is considered to be low risk. However, if TER < 10, risk must be assessed using long-term reproduction tests. When the possibility of long-term exposure to pesticides is foreseen (required when soil DT_{90} in field > 365 days or application of the pesticide exceeds 6 times per year, required case-by-case when DT_{90} = 100–365 days with the use 3–6 times, unnecessary when DT_{90} < 100 days and number of applications is less than three per year; DT_{90} being the period necessary for 90% degradation of the pesticide), reproduction tests also must be carried out. In reproduction tests, NOEC, which is the endpoint in risk assessments, and the initial value for PEC soil, which is calculated separately, are compared and TER (NOEC/PEC soil) is calculated; when TER is greater than 5, it is evaluated as being low risk. If TER < 5, safety is evaluated using options such as lab tests using natural soil and field tests.

(ii) Soil micro-organisms

The soil collected from pastures, etc., is treated with a test concentration equivalent to the maximum PEC soil of the pesticide, and it is evaluated as being low risk if the difference from an untreated control group is within 25% in 28 days to a maximum 100 days after treatment, with the indices being carbon mineralization and nitrogen transformation by microorganisms according to the amount of carbon dioxide produced and amount of nitrogen produced as nitrate.

(iii) Soil macro-organisms other than earthworms

When the standard test HQ ≥ 2 for non-target arthropods with soil DT_{90} in field = 100 to 365 days and long-term TER for earthworms < 5 or effect on soil microorganisms > 25% (versus an untreated group), risk is evaluated by reproduction tests carried out on springtails (*Folsomia candida*) or gamasid mites (*Hypoaspis aculeifer*). The NOEC and PEC soil obtained are compared, and the pesticide is evaluated as being low risk if TER (NOEC/PEC soil) is greater than 5.

When safety cannot be confirmed in tests with *Folsomia candida* or *Hypoaspis aculeifer* or when soil DT_{90} in field > 365 days, assessments are made using litter bag tests which have as an index the decomposition of organic matter from the standpoint of evaluating the effects on the cyclical change of materials. In these tests, a prescribed amount of straw is buried in an outdoor field to which a soil concentration (plateau concentration) of pesticide that reaches a stable level after long-term use calculated using simulated models has been applied. Subsequently, within one week, the maximum annual amount of pesticide is applied, and the assessment is made by comparing the reduction in straw weight over 6 to 12 months with an untreated group.

(Ⅴ) Non-target plants[8), 9), 15)]

Assessments are necessary other than for cases where exposure is negligible such as in the cases of rodenticides and seed treatment agents as well as applications inside greenhouses. Tiered assessment methods have been proposed with a first tier that uses initial screening information such as pesticide efficacy and phytotoxicity effects, a second tier that derives the 50% effect rate ER_{50} (g/ha) from dose-response tests using 6 to 10 species of plants and compares it with the application rate and a third tier based on field testing. Herbicides and plant growth regulators require second or higher tier assessments.

(Ⅵ) Assessment of metabolites[9)]

Risk assessments are necessary, based on the amounts of degradation products (metabolites) formed from the active ingredient (that is, the parent compound) in the pesticide formulation through biological and abiotic processes in the environment. Assessments through tests such as acute toxicity tests, are carried out on major metabolites found at levels of 10% or more in soil metabolism tests, but assessments of minor metabolites less than 10% are carried out using related information

Table 3 Data requirements and parameters for exposure/risk for the pesticide registration in US (Tier-1)

Taxonomic groups	Required data	Endpoints	Parameters for exposure	Parameters for risk	Level of concern
Birds	Acute: Acute oral study	LD_{50}	Dose-based EEC	Dose-based RQ	0.5 (0.1 for endangered species)
	Short term: 5-day dietary study	LC_{50}	Dietary-based EEC	Dietary-based RQ	
	Long term: Reproduction study	NOEC			1
Mammals	Acute: Acute oral study	LD_{50}	Dose-based EEC	Dose-based RQ	0.5 (0.1 for endangered species)
	Long term: Reproduction study (or Teratology study)	NOEC or NOEL	Dietary-based EEC or Dose-based EEC	Dietary-based RQ or Dose-based RQ	1
Bees	Acute contact study	LD_{50}	–	–	< 11 μg a.s./bee
Non-target plants	Seedling emergence study	NOEC	Application rate or 3 times EEC	–	< 25% adverse effect
	Vegetative vigor study				

RQ = EEC or Application rate/Toxicity endpoint, a.s. = active substance

such as hypothetical toxicity, for example, that assumes toxicity 10 times that of the parent compound.

(2) USA

We will give an overview of the guidelines for assessment indices and acceptable criteria[11), 12), 19)–21)] for the birds, mammals, bees and non-target plants targeted by EPA assessments for registration in the USA (**Table 3**). The basic concepts are the same as those for the EU, but those in the USA are characterized by consideration being given to endangered species. In addition, risk quotients (RQ: reciprocal of TER), which are predicted concentrations in the environment/endpoints are used in risk assessments.

(I) Birds and mammals[11), 12), 19)–22)]

According to the intended pattern of use of the pesticide, acute oral tests are required in two species of birds (one species of passerine and one species that is either bobwhite or mallard duck), and 5-day dietary and reproduction tests for two species of birds (bobwhite and mallard duck). Moreover, when liquid formulations are used in greenhouses or there is other use in greenhouses, acute oral test data is unnecessary. On the other hand, for mammals, acute oral tests conducted in rats in human health risk assessments and long-term test data such as that for two-generation reproduction tests are evaluated, and acute toxicity tests and other tests using wild species are required according to the results of these tests.

The toxicity test endpoints for birds and mammals used in risk assessments are LD_{50} for acute oral tests, LC_{50} for 5-day dietary tests (birds only) and NOEC or NOEL for reproduction tests. These endpoints are compared with the estimated environmental concentration (EEC), and the RQ (EEC/endpoint) is calculated and compared with the level of concern (LOC).

As with the EU, the USA has a different assessment scenario[22)] according to differences in treatment methods (foliar spray application with liquid formulation, granular application to the soil, seed treatment). Here, we will discuss foliar spray application. In acute oral assessments, dose-based EEC for foods such as short grass, seeds and insects adjusted according to body weight into small, medium and large sizes and adjusted LD_{50}, which adjusts the endpoint according to body weight, are compared. In short-term diet and long-term risk assessments, dietary-based EEC in birds and endpoints (LC_{50} and NOEC) are compared, and in mammals dose-based EEC and adjusted NOEL are also compared. When RQ from risk assessment is greater than the LOC, higher tier assessments will be carried out through a reduction in exposure levels by measuring the actual concentrations in the diet and refining the default values (half-life in food, etc.). When concerns about safety remain even then, risk assessments are carried out by performing further tests such as field tests.

(II) Bees[11), 12), 19)–21)]

Acute contact test data on the western honey bee (*Apis mellifera*) may be required according to the intended pattern of use of the pesticide. The endpoint used for risk assessments is LD_{50} (μg/bee), and when LD_{50} < 11 μg/bee, assessments through higher tier residue tests or field tests on pollinators are required.

(III) Non-target plants[11), 12), 19)–21)]

As with bees, seeding emergence and vegetative vigor tests positioned as tier-1 are required according to the intended pattern of use of the pesticide.

Tests are carried out with the actual application rate of or three times the EEC level, and if an effect of 25% or greater is found in comparisons with the untreated group, subsequent assessments are carried out using tier-2 tests that evaluate dose correlations or field tests for tier-3.

(3) Japan

The groups of organisms targeted by the data requirements[13] in Japan are birds, honey bees, natural enemy insects and silkworms, and while consideration may be given to industries connected with agriculture, no quantitative risk assessments are carried out for terrestrial ecosystems, and currently risk-management remains at warnings on product labels based only on hazard assessments.

(I) Birds[13), 23)]

Acute oral tests are carried out, and when strong toxicity (LD_{50} < 300 mg/kg) is found, dietary tests are carried out. When the toxicity is low, warnings are unnecessary.

(Ⅱ) Bees[13), 23)]

From the standpoint of risk assessments for both apiculture and pollinating insects, acute toxicity tests (acute oral or acute contact tests) are carried out on western honey bees. However, when there is thought to be no risk of honey bees being exposed to the pesticide in question because of the type of pesticide formulation or method of use (for example: granules or stored fumigants that are used only within a facility), there is an exemption from submitting test results. When strong toxicity is found in the results of acute toxicity tests, field tests are required. When LD_{50} is 11 μg/bee or greater or effects at maximum doses in pesticide registration applications are not found, warnings are not necessary.

(Ⅲ) Natural enemy insects[13), 23)]

Tests are required from the standpoint of assessing risks to natural enemies registered as pesticides or indigenous natural enemies. Three species of two orders are selected as test organisms from predator insects (Diptera, Hemiptera, Coleoptera and Neuroptera), parasitic wasps (Hymenoptera), Araneae and predatory mites (Acari), and acute toxicity tests are carried out at the maximum dose in the pesticide registration application. Field tests are required when strong toxicity is found in the results of the acute toxicity tests. When the toxicity is low, warnings are unnecessary.

(Ⅳ) Silkworms[13), 23)]

The acute oral toxicity tests are conducted by feeding mulberry leaves which have been applied at maximum dose in the pesticide registration application and then air dried. However, when it is found that there is no danger of exposure to the pesticide in question through ingestion by silkworms from the type of pesticide formulation and method of use (for example: granules or stored fumigants that are used only within a facility), there is an exemption from submitting test results. When a strong toxicity is found in the results of acute toxicity tests, residue tests are carried out by the feeding of leaves after a certain number of days have elapsed following treatment with the pesticide. When toxicity is low in the results of tests of the effects on silkworms, warnings are unnecessary.

3. Recent trends

If we look at this globally, even with the assessment systems introduced in Europe and the USA early on, there are a variety of problems with current evaluation systems and assessment methods because of the complexity of organisms and ecosystems, the large number of points that are unknown ecologically and the difficulty of assessing risks related to the results, and more research, investigations, improvements and proposals are being done on terrestrial ecological risk assessment methods. For example, the conventional bird and mammal risk assessment systems that have been extremely conservative using default values are being improved by the EU, and wide ranging lower tier assessment scenarios that combine the types and forms of target crops, methods of use for pesticides and seasons of application as well as ecological characteristics of the animal species that might be exposed have been introduced.[10] In addition, risk assessment methods are also being actively discussed in workshops such as ESCORT3,[24] ICPBR[25] and IRIS[26] for non-target arthropods, honey bees and soil organisms respectively, and work is being done on improving the EU risk assessment guidance documents for terrestrial organisms in general based on these trends.[27]

On the other hand, data requirements for pesticide registration are being revised, and in the USA, in a recent revision to the data requirements,[12] acute oral tests are now required for passerine species that directly

assesses the risk for songbirds. (At the time, there was a proposal for the red-winged blackbird to be the recommended species, but because of the difficulty in handling wild species, such as the necessity for capturing them, a specific name was omitted.) In the EU, mallard ducks were removed as a recommended species because of a possible underestimation by issues of regurgitation, and the 5-day dietary tests for birds were also removed from the standard test items because the ecotoxicological significance of the data obtained was unclear and for reasons of animal welfare.[10] In addition, there are ongoing deliberations on handling tests of functional aspects of soil ecosystems (soil micro-organism tests and organic decomposition tests), and there is a movement toward assembling test requirements for data on amphibians and reptiles.[28]

In the recent trends in regulations concerning endocrine disrupting (ED) chemical substances, the United States has started full-scale work with an endocrine disruptor screening program (EDSP) for probing whether or not there are ED actions in chemical substances, and as a part of this, tier-1 screening requirements were issued in 2009 and are in progress.[29]

In the EDSP, the main targets are to assess risks on the functions of estrogen, androgen and thyroid hormone using a two-tier test system with tier-1 screening and tier-2 testing. The tier-1 screening is made up of five *in vitro* tests and six animal (*in vivo*) tests for the purpose of detecting chemical substance action on the endocrine system in organisms. The tier-2 tests are to identify any harmful effects of the chemical substances in organisms, and currently their suitability is being verified. Among these, a system capable of highly sensitive detection of effects on thyroid function is also limited in a test for the effects on human health, and work is being done on tier-1 screening for an amphibian metamorphosis assay using frogs as an organism in the environment. There is an increasing importance for this test using the frogs not only from the standpoint of the specificity of the item being evaluated but also from that of the risks to amphibians, which have an important place in the terrestrial ecosystem. In addition, two-generation reproduction test for birds are being developed as a method for assessing risks to terrestrial organisms for tier-2 testing.[30]

On the other hand, in the EU, a regulation introducing standards for cut-off criteria preventing the marketing and use of chemical compounds that are highly risky for potential persistence in ecosystems, bioconcentration and ED action on the regulatory scheme for pesticide registration have been issued,[31] and these must be given consideration as new criteria separate from the quantitative risk assessments described above.

Examples of Higher Tier Tests and New Work in Terrestrial Ecological Risk Assessment

1. Examples of higher tier tests

There have been some concerns about the risks for terrestrial ecosystems from some of our pesticides because of innate biological activity as pesticides or environmental behaviour. However, we will introduce examples in the following of how their safety was demonstrated through the evaluation in the EU by carrying out strict higher tier tests using agricultural fields.

(1) Outdoor honey bee tests for pyriproxyfen

The acute toxicity of the insecticide pyriproxyfen for adult honey bees is extremely low (western honey bee, LD_{50} = 74 to greater than 100 μg/bee). In addition, in tests where artificial forage (pollen) treated with pyriproxyfen at the concentration of 25 ppm was fed to a colony, there was no effect on the larval development (emergence).[32] However, because this agent is an insect growth regulator classified as an juvenile hormone mimic, we thought that it was necessary to more strictly confirm whether or not there were risks to the growth and metamorphosis of honey bee larvae as predicted for actual situations. In this case, it was necessary to consider the special ecology as a social insect in which there are various internal roles in the colony for the queen bee and worker bees and a division of roles inside and outside the hive, regular development of larvae and a complex and, further, a well ordered energy cycle for honey and pollen which is the food.[33)–36)] In addition, since the situation of the honey source and its palatability for honey bees also vary according to the crops and growing conditions at sites of actual use of pesticides, these points also had to be considered. On the other hand, since there are no detailed test guidelines for field tests focusing on the growth of honey bee larvae, we drew a test design with reference to internationally standard test methods[37), 38)] for honey bees. In other words, we used the standard plant *Phacelia tanacetifolia*, which has palatability for honey bees and carried out field assessments of risk using colonies during the period it is in full bloom to examine the effects of this agent (**Table 4**, **Fig. 4**, **Fig. 5**). Based on the biological activity of pyriproxyfen, the complex energy

cycle within the colony and the instability of field testing, the assessment period was extended to two months after treatment with the agent from the standard field test method of 1 to 2 weeks.[37), 39)] During the monitoring period, the colony status and larval development were evaluated by observing the foraging worker bees, harvesting activities and mortality rate, as well as the larvae growth cycle. A honey bee colony is kept at a constant temperature by the worker bees, and there is a fixed period from egg to adult emergence.

It is known that daughter bee eggs laid by the queen bee hatch in three days; the post-hatch larvae are fed by nurse worker bees and after six days, they pupate and the cell where the larva was is capped. Adult emergence takes place 12 days later.[35)] Based on this cycle of development, areas of cells housing eggs or larva in the hive frame were specified in advance using insect pins (**Fig. 6**), and development of the larvae in individual cells was observed for two cycles to strictly assess the effects on larval development.

As a result, both the eggs and the larvae identified in the comb were found to develop into healthy adult bees in the same manner as those in the control hives that had not been treated with pyriproxyfen (**Fig. 7**). As an

Table 4 Outline of the honey bee field test for pyriproxyfen focusing on brood and colony health

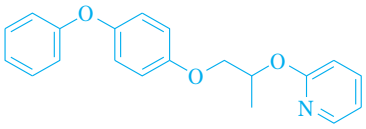
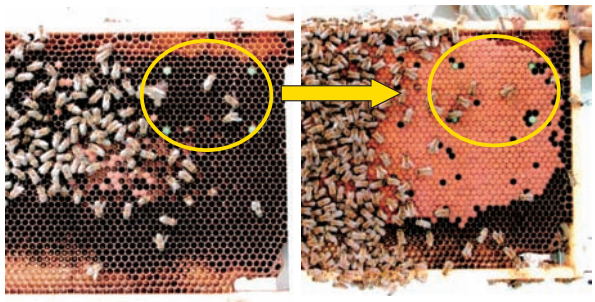
Test Organism:	Honeybee (*Apis mellifera*)
Test Unit:	Colony (ca. 60,000 – 80,000 worker bees)
Field site:	Germany, 0.5 ha/group, 3 km distance full flowering *Phacelia tanacetifolia* (bee attractive)
Test substance:	Pyriproxyfen 10%EC, active substance: Pyriproxyfen (insect growth regulator) 
Test group:	treatment (75 g a.s./ha) and control, 4 colonies/group
Observation:	bee flight activity, foraging intensity, dead bees (hive and field), brood development, frame conditions, hive weight

Fig. 4 Photograph of the test substance treatment to the testing field

Fig. 5 Photograph of the honey bee hives set beside the testing field with dead bee traps in front of their entrances



Marked larval cells in the pre-exposed colony at Day -1

Development of larval cells into capped cells (pupae) in the pyriproxyfen treatment group colony at Day 7

Fig. 6 Photograph of the individual monitoring of honey bee brood development by marking cells in the comb

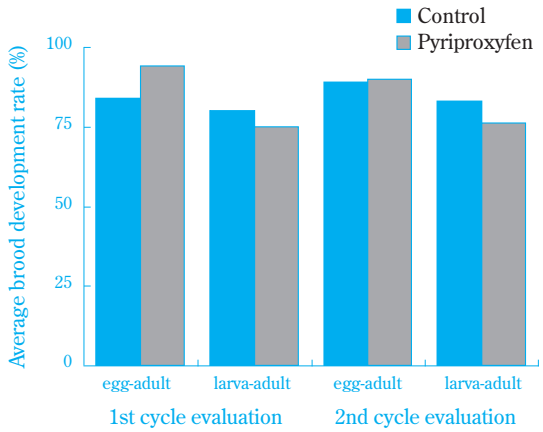


Fig. 7 Honey bee brood development monitored by individual cells in the field test for pyriproxyfen

example, **Fig. 6** shows the changes in the cells housing larva from before the application to the following week in the hive treated with pyriproxyfen. In the cells where larvae were observed at the first observation, capped cells can be found showing that the pupation occurred as it should in the following week.

In addition, an acquired trait of honey bees is to clear out the corpses of the dead within the colony through the entrance to the hive, including larvae that have not emerged as adults.[37), 39)] The colony reacts in a sensitive manner to environmental changes such as movement of the hive, and it is known that directly after the hive is moved, many dead individuals are temporarily carried to the neighborhood of the hive entrance.[37)] Therefore, to eliminate the bad effects of such stress factors on the test evaluations, a test design was employed where the agent was applied after several days of acclimation following the moving of the hive to the test site. In addition, a dead bee trap (device for collecting the dead individuals eliminated from the hive, red container in **Fig. 5**) was installed at the entrance to the hive for confirmation, and the number of dead individuals was observed. The number of dead individuals from the colony after treatment with pyriproxyfen obtained by continuous monitoring of the trap was clearly at a lower level than that prior to treatment (during acclimation), and was the same as for the untreated colonies. We found that there was no increase in the number of dead individuals because of the pyriproxyfen treatment under conditions where there was no burden of colony stress.

Furthermore, even with the weight of the hive, which was measured over the course of the time, absolutely no difference was seen between the pyriproxyfen treated group and the untreated group, and a healthy seasonal increase in weight was found (**Fig. 8**).

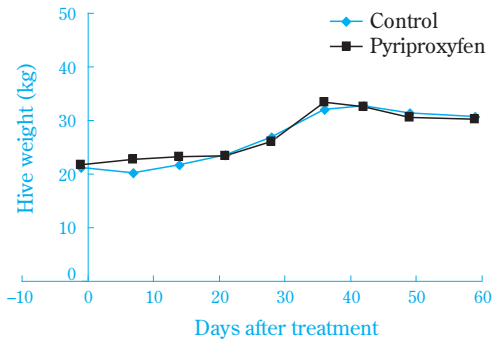


Fig. 8 Hive weight changes monitored during the field test for pyriproxyfen

From these results, we were able to verify that the possibility of pyriproxyfen affecting honey bee larval development and colonies was low even with realistic exposure conditions in the field.

(2) The field testing of esfenvalerate on non-target arthropods

This differs from the natural environment; the deviations in density of the vegetation are adjusted, and human intervention such as weeding and application of fertilizer occurs. It is thought that these factors affect the variations in groups of individual arthropods inhabiting agricultural fields. In EU non-target arthropod risk assessments related to this, the allowable assessment standards differ for direct in-field application of pesticides and possible off-field exposure to minute amounts because of pesticide drift, which is an immigration source for organisms in the field. The former is regulated by recovery properties within one year, and the latter by recovery properties within the period that is ecologically permissible.[9), 18)]

Esfenvalerate, which is a pyrethroid insecticide, affects some standard test organisms in higher tier tests for insecticidal properties carried out in the laboratory, and since it did not reach the allowable standard in assessments of the risks, we decided to conduct outdoor risk assessment tests to confirm the safety of this agent. In carrying out field tests, we decided to evaluate the effects on non-target arthropods under conditions applied in orchards, taking into consideration worst-case conditions along with the number of applications and amount applied for the EU registration. Furthermore, to precisely carry out evaluations for the off-field habitat, which differed from the allowance standards, we provided test groups for 7.5 and 1.5 g a.s./ha envisioned for spray drift in addition to the application rate of 15 g a.s./ha. To evaluate groups of individuals, we incorporated observation items for predatory mites, aphid predators, parasitic species and other predators as well as spider mites and aphids, which are food organisms and target organisms, and considered the evaluation of indirect effects due to insufficient food (**Table 5**, **Fig. 9**).

Fig. 10 shows examples of the species observed. While a small decrease was found in the 7.5 and 15 g a.s./ha treatment groups after applications when compared with the number of individual predatory mites which varies seasonally in untreated control groups, the number of individuals recovered to the same level as

Table 5 Outline of the apple field test for esfenvalerate evaluating effects on non-target arthropods

Test Organism:	Natural arthropods in orchard field
Field site:	France, apple field, ca. 81 m^2/plot × 4 plots/group
Test substance:	Sumi-Alpha® 5EC, active substance: Esfenvalerate (pyrethroid insecticide)
Test group:	treatment (1.5, 7.5, 15 g a.s./ha) and control, triplicate treatments (2 weeks interval)
Observation:	abundance of arthropods by appropriate methods (leaf sampling, beating tray, visual check); predatory mite & their eggs, aphid predators (e.g. lacewing, ladybird beetle, predatory bug), parasitic wasps, other predators (e.g. spider), prey (i.e. spider mites & aphids)

Fig. 9 Photograph of the test substance treatment by hand-held sprayer in an apple orchard

the untreated control groups within one month, and no effect was found in the period of observation in the 1.5 g a.s./ha test group (**Fig. 11**). In addition, a sufficient number of predatory mite eggs was counted in observations at the same time, and we were able to confirm that the number of individuals recovered because of the ability to continuously reproduce.

As with the predatory mites, a clear decrease in the number of individuals was found in groups of individual aphid predators such as lacewings in the 7.5 and 15 g a.s./ha treatment groups compared with the untreated control groups, but after low numbers of individuals with seasonal changes that included the untreated control groups in midsummer, the difference with the untreated control groups disappeared, and it was found that around one month after application, the number of indi-

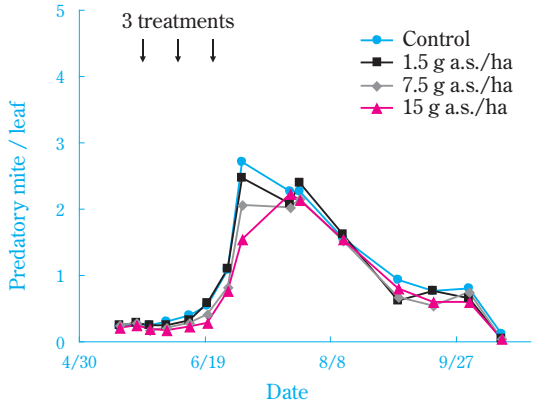


Fig. 11 Number of predatory mites during the apple orchard field test for esfenvalerate

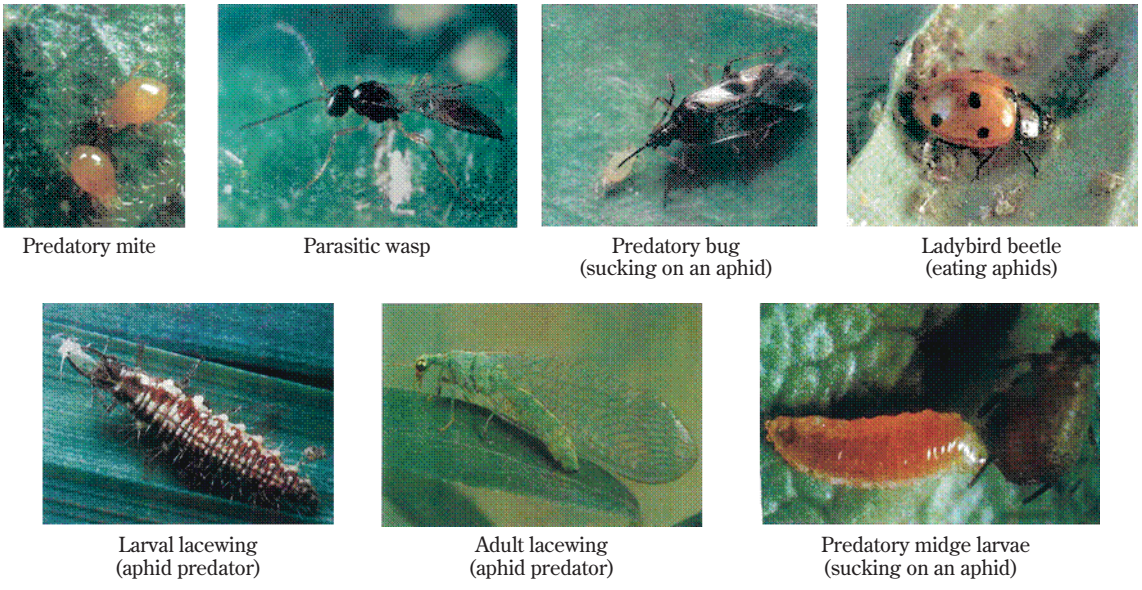


Fig. 10 Photographs of the typical arthropods observed in the apple orchard field test for esfenvalerate

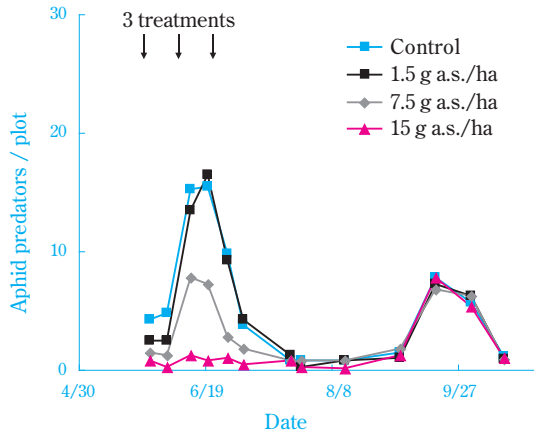


Fig. 12 Number of aphid predators during the apple orchard field test for esfenvalerate

viduals recovered (**Fig. 12**). In addition, that change also matches the reduction in number of individual aphids, which are a food organism and a target harmful insect, and in light of the low toxicity data for lacewings in semi-field tests carried out separately, the possibility is suggested that the reduction in the number of individual aphid predators directly after application is an indirect effect of a food (the pest, aphids) insufficiency rather than a direct effect of treatment with esfenvalerate.

From the results of assessing various organisms as shown in **Table 6**, the test area for the 1.5 g a.s./ha envisioned for exposure due to drift in the area around the field had no effect. Even with three applications at 15 g a.s./ha, which was the worst-case application condition and the condition for the number of applications for use in the EU, recovery of all taxa was found within one month, and we were able to confirm the safety for non-target arthropods.

Table 6 Summary results of non-target arthropods in the apple orchard field test for esfenvalerate

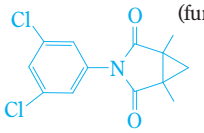
	Esfenvalerate treatment		
	1.5 g a.s./ha	7.5 g a.s./ha	15 g a.s./ha
Predatory mites	No effect	Recover within 1M	Recover within 1M
Spider mites	No effect	Recover within 1M	Recover within 1M
Aphid predators	No effect	Recover within 1M #	Recover within 1M #
Other predators	No effect	Recover within 1M #	Recover within 1M #
Parasitic wasps	No effect	Recover within 1M #	Recover within 1M #

M: month, #: possibly indirect effect due to food limitation

(3) Assessment of organic decomposition function of soil (litter bag tests) for procymidone

In standard laboratory tests on soil organisms, the effects of the fungicide procymidone on all groups of organisms was low indicating results of 14d-LC_{50} > 1000 mg a.s./kg, 56d-NOEC = 3.750 kg a.s./ha (equivalent to 5 mg a.s./kg) in earthworms and effect level less than 25% on micro-organisms > 10 mg a.s./kg and thus, it is considered that concern for the effects on soil organisms was low based on this data. In addition, the actual number of applications per year is few, and even though a soil DT_{90} in the field was less than one year (349 days, n = 3), the possibility of the soil persistency varying somewhat depending on soil properties was suggested. Therefore, to confirm the safety for actual soil ecosystems, we carried out specific outdoor tests using bags with straw, the so-called litter bag test, for evaluating the material cycle in the soil, which is positioned as a higher tier test in the EU registration scheme (**Table 7**).

Table 7 Outline of the litter bag test for procymidone evaluating organic matter breakdown

Test Unit:	Litter bags (4 g of dry wheat straw)
Field site & Season:	Switzerland, 25 ~ 30 m^2/plot × 4 plots/group, May ~ November
Test substance:	KIMONO® 50SC, active substance: Procymidone (fungicide) 
Test group:	treatment (3.752 kg a.s./ha, 2.5 times annual rate) and control
Treatment:	spray the litter bags on the test field, buried in the soil at 5 cm depth & marked the position
Measurement:	Ash free weight of dry residue of straw (1, 3 and 6 months after treatment)

This is a test that assesses the effect on the decomposition of a straw bundle buried in the soil, and it is a unique test focusing on the organic decomposition function of soil organisms in an agricultural field. It started originally in the 1950s as a soil ecological research method, and various researchers have published comparisons with different conditions including types of fallen leaves and forms of storage.[40] In general, contributions are made to the decomposition of organic matter in the soil not only by soil micro-organisms but also by soil meso-fauna (body length: approximately 0.2 to 2

mm) such as springtails and mites and soil macro-fauna (body length: approximately 2 to 20 mm) such as earthworms and millipedes. In addition, not only is there direct decomposition by organisms, but also there is a complex relationship of various factors contributing indirectly due to conditional relationships such as soil plowing and agitation by earthworms.[40] From these points of view, the organic decomposition properties of the soil have two important aspects for the ecosystem, structure and function, and of these, an assessment that focuses on the latter is aimed at. In the litter bag test, a nylon mesh bag with the straw bundle in it is buried in the soil along with soil that has been spray treated with the test item (**Fig. 13**); the buried bags are sampled over time, and the residual content of the straw in the litter bag is quantified from the combustion ash to assess the decomposition properties.[9), 41)]

Fig. 13 Photograph of the test field prepared with nylon mesh bags containing litter

The rates of straw decomposition over time in the untreated and procymidone treated groups are shown in **Fig. 14**. No difference was found in the two test groups even six months after treatment with 70% decomposition and thus, it was confirmed that there were no problems with the continued function of the soil ecosystem even in an agricultural field under actual use.

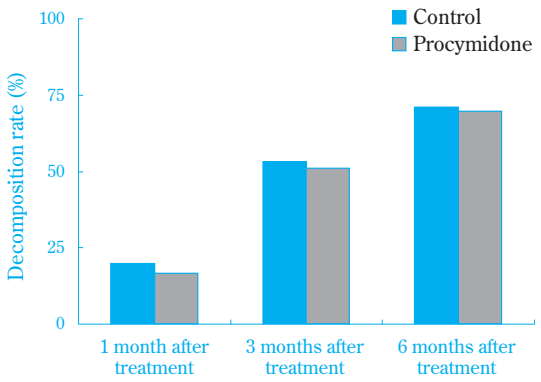
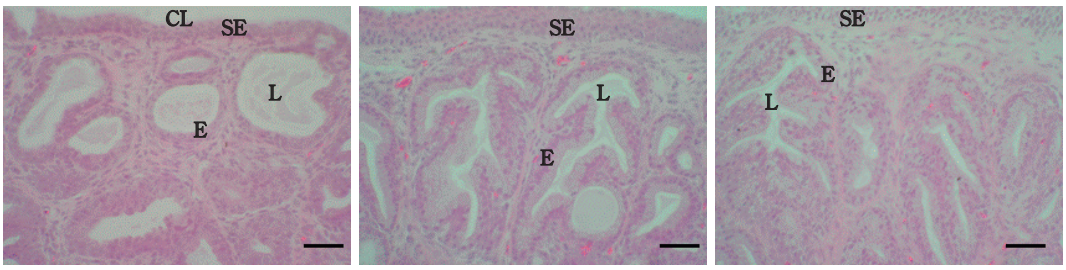


Fig. 14 Decomposition of straw in the litter bag test for procymidone

2. Examples of new work – Establishment of a test system for detecting ED potential of chemicals with the sensitive avian embryonic endpoint –

Besides carrying out the EDSP in the USA, there is a worldwide strengthening of regulations concerning ED issue such as the data requirements in the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) regulations and the pesticide registration regulations in Europe as well as the ED problem investigations dating from SPEED98 in Japan. Under these circumstances, the OECD has been proceeding to develop various test methods for detecting and characterizing potential ED chemicals, and as part of this, the development of avian two-generation reproduction test methods for birds has been continuing. On the other hand, there have been investigations into developing screening assays in birds, although less research has been focused on understanding potential effects of androgenic xenobiotics in birds compared with estrogenic-related substances. The unique method for detecting the androgenic effects of chemicals in birds is an *in vivo* assay using mature animals; therefore, there are many problems including securing of facilities for rearing, specific test operations and variations in test results in the *vivo* assay. If there was a screening assay that could detect the ED potential of chemicals for birds quantitatively, it would be useful for designing a definitive test to obtain an understanding of the ED effect as well as grasping the characters of chemicals at an early developmental stage. Therefore, the aim of this work was to establish a screening system for detecting androgenic and anti-androgenic potential of chemicals in birds.

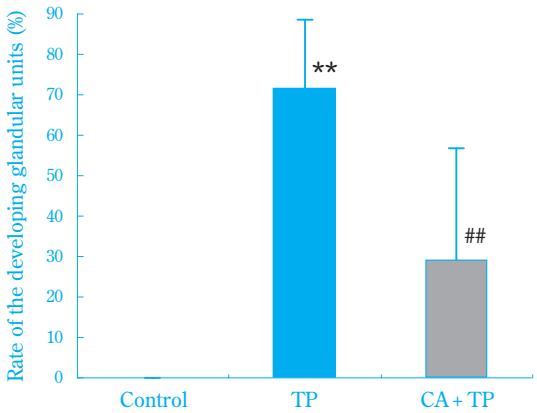
The tissue structural changes in the cloacal gland that is in the anus area of quail embryos was on the focus of the endpoint of androgenic effects, and fertilized Japanese quail embryos were injected with cyproterone acetate (CA), an anti-androgenic compound, on d 12 of incubation, following by injection of testosterone propionate (TP), androgenic compound, on d 13 and histological examination on d 16. As a result, when TP was administered (TP group) the cloacal glandular cells in the TP-group showed a developed appearance with a tall height and inclusion of a mucous substance in the cytoplasm, whereas the injection with CA before TP (CA + TP group) suppressed the development of the cloacal



Control TP CA + TP

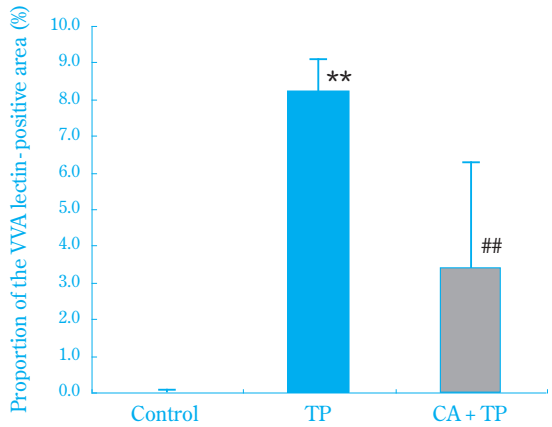
Hematoxylin and eosin staining. Scale bars = 30 μm.
CL = cloacal lumen, E = glandular epithelium, SE = surface epithelium, L = glandular lumen

Fig. 15 Sections of cloacal glands of Japanese quail embryos treated with corn oil (control), 300 μg of testosterone propionate (TP) or 75 μg cyproterone acetate and testosterone propionate (CA + TP)



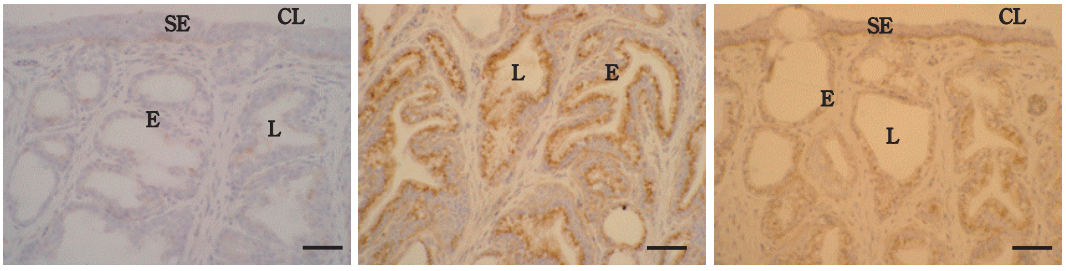
Values are mean ± SD (n = 10) of the ratio (%) of the number of developing glandular units to the total number of glandular units.
** : Significantly different from control (P < 0.01)
: Significantly different from TP group (P < 0.01)

Fig. 16 Effects on corn oil (control), 300 μg of testosterone propionate (TP) or 75 μg cyproterone acetate and testosterone propionate (CA + TP) on the development of the cloacal gland in Japanese quail embryos



Values are mean ± SD (n = 8) of the ratio (%) of the VVA lectin-positive area in the unit square of the cloacal gland.
** : Significantly different from control (P < 0.01)
: Significantly different from TP group (P < 0.01)

Fig. 18 Effects on corn oil (control), 300 μg of testosterone propionate (TP) or 75 μg cyproterone acetate and testosterone propionate (CA + TP) on the appearance of VVA lectin-positive substances in the cloacal glandular cells in Japanese quail embryos



Control TP CA + TP

Scale bars = 30 μm.
CL = cloacal lumen, E = glandular epithelium, SE = surface epithelium, L = glandular lumen

Fig. 17 Sections of cloacal glands of Japanese quail embryos treated with corn oil (control), 300 μg of testosterone propionate (TP) or 75 μg cyproterone acetate and testosterone propionate (CA + TP) stained by *Vicia villosa* (VVA) lectin

gland (**Fig. 15**). These results suggest that the ratio of developing glandular units could be used quantitatively for evaluation of the androgenic and anti-androgenic effects of compounds (**Fig. 16**).[42]

Next, we focused on the secretions of mucopolysaccharides accompanying maturation of the cloacal glands, and examined whether the lectins, which have binding capacity for specific sugar chains, exhibited a positive reaction in the cytoplasm of the cloacal gland cells developed in response to androgen. Injection with CA was performed on d 12 of incubation, following by TP injection on d 13 and examination by lectin histochemistry on d 16. Among the 14 lectins, one type showed the strongest binding signals in the cytoplasm of developing cloacal gland cells simulated by TP, whereas the injection with CA before TP (CA + TP group) suppressed the increase of binding signals (**Fig. 17**). These results suggest that the current method is applicable for detecting the androgenic and anti-androgenic effects of compounds more objectively than simple histology, because the lectin-labeling density could be analyzed using computer-assisted image analysis (**Fig. 18**).[43]

Because the same results were obtained in the tests using different chemicals, we suggest that the test system for androgenic and anti-androgenic ED potential of chemicals concerning the nuclear androgen receptors could be useful for evaluation of various chemicals. The current system also enabled the test to be performed in a usual laboratory system without any special facilities and equipment, specifically no surgical treatment of animals is necessary unlike the methods described by other research groups.

Future Outlook

Risk assessments for pesticides in terrestrial ecosystems are complicated and difficult, and every day there are major improvements with the progress in science at the regulatory agencies in the EU and USA that are carrying out detailed ecological risk assessments. The OECD test guidelines have reached 30 methods for ecological risk assessments, and even now, new test types and test methods for standard laboratory tests are being investigated. On the other hand, with the pesticide regulations in Japan, there is a movement to introduce risk assessments that compare toxicity indices and exposure levels in two terrestrial ecosystem assessments in consideration of methods in use in the EU and USA.[44), 45)] It is well understood that the assessment methods in the EU and USA have been established for the ecosystems in those areas, and it is desirable to interpret scientific assessment methods suitably and construct realistic risk assessment scenarios that reflect the ecosystems peculiar to Japan. Under such circumstances, risk assessments of the affects on honey bees are becoming more and more important in the field of terrestrial risk assessments because of the increase in worldwide concern. Along with detailed analysis of the toxicity expressions for honey bees, we must consider the various routes, periods and concentrations for honey bee exposure, and then carry out comprehensive risk assessments that always grasp and are driven by the most recent related knowledge and regulatory agency evaluations. In addition, the use of amphibians, which have been brought up as an index species for organisms in terrestrial environments up to now, and among them frogs, in risk assessments is becoming important, and construction of test systems for this is a problem to be investigated in the future.

In the future, we must work on accumulating greater know-how and techniques, including the simple assessment system using bird embryos which has been introduced as an example of new work, and powered by these, we want to confirm the safety of Sumitomo Chemical pesticides for the ecosystem and to develop pesticides that are more environmentally benign.

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