United States Environmental Protection Agency

Technical Overview of Ecological Risk Assessment - Analysis Phase: Ecological Effects Characterization

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The analysis phase examines two major parts of risk, exposure and effects, and their relationship with each other. The process for examining effects is called **ecological effects characterization**, whereas the process for examining exposure is called **exposure characterization**. During the analysis phase, risk assessors:

- select data that can be used to characterize exposure and ecological effects;
- characterize exposure by examining sources of the pesticide(s) or stressors, distribution of the pesticides in the environment, and extent of contact with pesticides;
- characterize effects by examining stressor-response relationships, evidence for cause and effect, relationship between measures of effect and assessment endpoints; and
- summarize conclusions about exposure and effects.

About Ecological Effects Characterization

An **ecological effects characterization** describes how toxic a pesticide is to different organisms and/or to other ecological entities (e.g., community), what effects it produces, how the effects relate to the assessment endpoints, and how these effects change with varying levels of pesticide exposure. This

characterization is based on a **stressor-response profile** that describes how toxic a pesticide is to various plants and animals, the cause-and-effect relationships, how fast the organism(s) recovers, relationships between the assessment endpoints and measures of effect, and the uncertainties and assumptions associated with the analysis. The stressor-response profile is the final product of the ecological effects characterization.

EPA estimates the toxicity or hazard of a pesticide by evaluating ecological effects tests that vary from short-term (acute) to long-term (chronic) laboratory studies and may also include field studies. In these tests, animals and plants are exposed to different amounts of pesticides, and their responses to these varying concentrations are measured. The results of these tests may be used to establish a dose-response or cause-and-effect relationship between the amount of pesticide to which the organism is exposed and the effects on the organism.

In most cases, toxicity tests are conducted on an active ingredient basis. If formulated product effects data are available, they will also be considered in the risk assessment. In addition, data on degradates of potential toxicological concern will be incorporated into the risk assessment.

In this testing system, surrogate or substitute organisms are used to represent a group of organisms. For example, the laboratory rat may be used to represent all mammalian species.

Some of the impacts or ecological effects that are measured in ecotoxicity tests include:

- mortality,
- reduction in growth,
- reproductive impairment,
- changes in numbers of species,
- bioaccumulation of residues in non-target organisms, and
- disruption of community and ecosystem-level functions.

For screening-level assessments, the following toxicity endpoints are used to calculate risk:

Aquatic Animals Toxicity Endpoints
for screening-level risk assessments

Assessment Type	**Endpoint**
Acute assessment	Lowest tested EC_{50} or LC_{50} for freshwater fish and invertebrates and estuarine/marine fish and invertebrates from acute toxicity tests
Chronic assessment	Lowest NOAEC for freshwater fish and invertebrates and estuarine/marine fish and invertebrates from early life-stage or full life-cycle tests

Aquatic toxicity endpoints for specific pesticides can be found at the following website: Office of Pesticide Programs' Aquatic Life Benchmarks

Terrestrial Animals Toxicity Endpoints
for screening-level risk assessments

Assessment Type	Endpoint
Acute avian assessment	Lowest LD_{50} (single oral dose) and LC_{50} (subacute dietary)
Chronic avian assessment	Lowest NOAEC for 21-week avian reproduction test
Acute mammalian assessment	Lowest LD_{50} from single oral dose test
Chronic mammalian assessment	Lowest NOAEC for two-generation reproduction test

Plant Toxicity Endpoints
for screening-level risk assessments

Plant Type	Endpoint
Terrestrial non-endangered	Lowest EC_{25} values from both seedling emergence and vegetative vigor for both monocots and dicots
Aquatic vascular and algae	Lowest EC_{50} for both vascular and algae
Terrestrial endangered	Lowest EC_5 or NOAEC for both seedling emergence and vegetative vigor for both monocots and dicots

Other toxicity endpoints may be used if professional judgment and lines of evidence determine that they can be linked to assessment endpoints in a reasonable manner. Guidance for using non-definitive endpoints in evaluating risks to animal species (listed and non-listed) can be found at: Guidance for Using Non-Definitive Endpoints in Evaluating Risks to Listed and Non-listed Animal Species.

The toxicological tests for ecological risk assessments are conducted under approved Harmonized Test Guidelines and Good Laboratory Practices Standards.

The results of these tests may be used to determine the need for mitigation measures that will minimize potential harmful effects to non-target organisms.

Ecological Effects Studies

The website, Data Requirements for Pesticide Registration, (Code of Federal Regulations - 40CFR Part 158: Subpart G 158.630 and 158.660) specifies the types and amounts of data that EPA may require to determine the risks of a pesticide to non-target terrestrial and aquatic animals and plants. The types of data needed may vary depending on where and how the pesticide is used. Individual studies that the Agency may require in support of the registration or approval of certain pesticides are listed below. In addition to registrant-submitted data, the

Agency may use toxicity endpoints found in publicly available literature for ecological risk assessments. To identify open literature studies that may potentially be incorporated into the Agency's ecological risk assessments, EPA uses the ECOTOXicology (ECOTOX) Database as a search engine. Guidance for evaluating ecological toxicity data in the open literature can be found at: Evaluation Guidelines for Ecological Toxicity Data in the Open Literature.

Terrestrial Animals

- **Birds**

  EPA may require the following acute oral, subacute dietary, and chronic tests in birds:

  - **Avian Acute Oral Toxicity** test is conducted with either an upland game bird (e.g., bobwhite quail) or a waterfowl species (e.g., mallard duck) and a passerine species (e.g., songbird). It is an acute, single-dose laboratory study that is designed to determine the amount of pesticide that will cause 50% mortality (LD_{50}) in a test population of birds. EPA's test guideline 850.2100 (21 pp, 250 K, About PDF) provides guidance on how to conduct avian acute oral toxicity tests. Since there are particular issues related to conducting this test with passerine species, additional guidance has been developed for EFED scientists for Guidance for Reviewing OCSPP 850.2100 Avian Oral Acute Toxicity Studies Conducted with Passerine Birds and Guidance for Use When Regurgitation is Observed in Avian Acute Toxicity Studies with Passerine Species. The agency has also developed Guidance for Classifying Studies Conducted Using the OECD Test Guideline 223 (TG223).

  - **Avian Subacute Dietary Toxicity** test is conducted with an upland game bird (e.g., bobwhite quail) and a waterfowl species (e.g., mallard duck). It is an acute, eight-day dietary laboratory study designed to determine the amount of pesticide that will cause 50% mortality (LC_{50}) in a test population of birds.

  - **Avian Reproduction** test uses both an upland game bird and a waterfowl species. It is a laboratory study (usually 20 weeks) designed to determine the amount of pesticide that will harm the reproductive capabilities of a test population of birds. Reproductive impairment is measured in terms of number of eggs laid per hen, number of cracked eggs, number of viable embryos, live three-week embryos of viable embryos, normal hatchlings of live three-week embryos, number of 14-day-old survivors. This test is used to determine the NOAEC (No Observed Adverse Effects Concentration) or LOAEC (Lowest Observed Adverse Effects Concentration) for the above parameters.

  - **Simulated or Actual Field Testing** is conducted either to quantify the actual risks in the field or to show that risks in the field under actual use are different than in the laboratory.

- **Non-Target Insects**

  EPA may require testing for effects in insect pollinators, such as honey bees, when the typical end-use product (TEP) is intended for outdoor use and honey bees may be exposed to the pesticide.

  - **Honey Bee Acute Contact Toxicity** is an acute, single-dose laboratory study designed to determine the quantity of pesticide that will cause 50% mortality (LD_{50}) in a test population of bees.

- **Honey Bee Toxicity of Residues on Foliage** is a laboratory test designed to determine the length of time over which field-weathered foliar residues (residues on leaves) remain toxic to honey bees.

- **Field Testing for Pollinators** may be required if the above tests indicate adverse effects on insects.

In January 2011, EPA participated in a SETAC Pellston workshop, which was designed to identify test methods for measuring exposure and toxic effects of systemic and non-systemic pesticides to pollinators and to identify a risk assessment process for pollinators. After the workshop was held, EPA worked with Health Canada's Pest Management Regulatory Agency and with the California Department of Pesticide Regulation to develop Guidance for Assessing Pesticide Risks to Bees. The guidance document describes the basic framework of the risk assessment process for honey bees and the data used to inform the various tiers of refinement that may be required to support the risk assessment process.

- **Mammals**

Generally, EPA scientists use the following studies to evaluate effects on small mammals:

- **Mammalian acute** test is an acute, single oral dose effects test conducted in laboratory rats and mice. This test measures the amount of pesticide that will produce mortality in 50% of the test animals (LD_{50}).

- **Mammalian subacute** test measures subacute dietary effects in rats and mice, i.e., the amount of pesticide in the diet that will produce mortality in 50% of the test animals (LD_{50}).

- **Mammalian chronic** tests measure reproduction and developmental effects in mice and rats. These tests include a two-generational reproductive study and a short-term teratogenic/developmental study.

Aquatic Animals

EPA may require acute and chronic effects testing in fish and invertebrates. The number and types of tests required depends on the use of the pesticide, the pesticide properties and in some cases, the results from previously conducted effects testing. These tests are typically conducted in the laboratory, with field tests being reserved for special cases to address specific uncertainties identified with laboratory testing. A brief summary of the different types of aquatic animal effects tests is provided below.

- **Acute (short-term) Tests**

- **Freshwater Fish Acute Toxicity** test uses both a cold water (*e.g.,* rainbow trout) and warm water (*e.g.,* bluegill) species. It usually lasts 96 hours and is designed to determine the concentration in water required to cause 50% lethality (LC50) in a test population of fish. (OSCPP 850.1075)

- **Freshwater Invertebrate Acute Toxicity** test uses a freshwater invertebrate (*e.g., Daphnia* sp.) in an acute, 48-hour laboratory study to determine the concentration of pesticide in water that causes 50% lethality (LC_{50}) or immobilization (EC_{50}) in a test population of invertebrates. (OSCPP 850.1010; 850.1020)

- **Estuarine and Marine Organisms Acute Toxicity** tests use marine/estuarine fish, shrimp, and mollusc species with exposure durations from 48 to 96 hours. They are designed to determine the concentration of pesticide that will cause 50% lethality (LC_{50}), incomplete shell growth (EC_{50}, oyster larvae), or reduced shell growth (EC_{50}, oyster juvenile/adult). (OSCPP 850.1025; 850.1035; 850.1045; 850.1055; 850.1075)

- **Bioconcentration, Bioavailability, Biomagnification Toxicity** tests are conducted with aquatic organisms (usually fish) to estimate the potential of a pesticide to accumulate in the tissue(s) of the organism under controlled laboratory conditions. These studies provide information on the degree of bioconcentration and/or biomagnification of a pesticide and the degree to which a pesticide's accumulation level can be reversed (depuration) should levels in the surrounding aquatic environment be reduced. (OSCPP 850.1710; 850.1730; 850.1850)

- **Chronic (long-term) Tests**

  - **Fish Early Life-Stage** test is designed to determine the amount of pesticide that will adversely affect hatching, survival, growth and early development of a test population of fish. Impaired survival, growth and development are measured in terms of number of embryos hatched, time to hatch, mortality of embryos, time to swim-up, and growth- weight and length. (OSCPP 850.1400)

  - **Fish Full Life-Cycle** test is designed to determine the amount of pesticide that will adversely affect the survival, growth and reproduction a test population of fish resulting from exposure throughout its entire life cycle. Impaired survival, growth and reproduction are measured in terms of the number of days to complete hatching, number of eggs produced and embryos hatched, number of surviving larvae hatched, length and weight of survivors among other endpoints. (OSCPP 850.1500)

  - Adjusting Fish Chronic Endpoints for Light-Dependent Peroxidizing Herbicides. Certain herbicides have been shown to exhibit enhanced toxicity to fish in the presence of ultraviolet light compared to toxicity observed under standard laboratory lighting. This class of herbicides is called light-dependent peroxidizing herbicides (LDPHs). To address concerns that standard laboratory tests may underestimate the toxicity of LPDHs in shallow, clear waters, EPA has developed guidance for identifying and evaluating these pesticides. This guidance involves applying a molar threshold value to fish chronic toxicity test endpoints in the absence of chemical-specific data to account for ultraviolet light-enhanced toxicity.

  - **Aquatic Invertebrate Life Cycle** test is designed to determine the amount of pesticide that will adversely affect the reproductive and developmental capabilities of a test population of aquatic invertebrates resulting from exposure throughout its entire life cycle. Typical test organisms include *Daphnia* and mysid shrimp. Impaired reproduction and development are measured in terms of survival, number of young produced, and growth and developmental rate.

  - **Whole Sediment Invertebrate Toxicity** testing may be conducted when the pesticide is expected to partition extensively to sediment particles. These test include shorter-term (10-day) and longer term (life cycle) exposures of freshwater and estuarine/marine aquatic invertebrates in whole sediment. The choice of test duration depends on the pesticide properties (*e.g.,* persistence) and other

considerations. Typical test organisms include the freshwater amphipod (*Hyalella azteca*), the freshwater midge (*Chironomus dilutus*) and the estuarine amphipod (*Leptocheirus plumulosus*).

- **Higher Tier Testing**
    - **Simulated or Actual Field Testing** may be conducted to quantify the actual risk in the field or to determine if effects observed in the field differ from those observed in the laboratory under comparable exposures.

Amphibians and Reptiles

In general, EPA uses bird toxicity data as a surrogate for terrestrial-phase reptiles and amphibians and fish toxicity data as a surrogate for aquatic-phase amphibians.

Non-Target Plants

In general, most pesticides are tested at the Tier I level for potential effects to both terrestrial and aquatic plants. EPA uses plant toxicity data to screen pesticides for their potential to move from the treated field to other crops or non-target plants. Currently only five aquatic plants and ten terrestrial crop plants are tested under EPA's non-target plant toxicity guidelines. The plant testing scheme is tiered, such that a limit concentration may be used in the first level. If the first level tests show effects, then additional tests are conducted at a higher level. In these tests, multiple species of aquatic plants (algae and duckweed) are tested for effects on growth (EC_{50}), and multiple species of herbaceous plants (crop species) are tested for seedling emergence and vegetative vigor (NOAEC and EC_{25}).

EPA scientists use the model TerrPlant, version 1.2.2 to provide screening-level estimates of exposure to terrestrial plants from single pesticide applications. TerrPlant estimates exposure to terrestrial plants in both dry and semi-aquatic areas from runoff and spray drift.

- **Tier I**
    - **Non-Target Area Terrestrial Plant Phytotoxicity** is a greenhouse or growth chamber test that consists of two parts: a test for seedling emergence and a test for vegetative vigor. Seedling emergence is a 14-21 day test conducted at one dose level. With this test the % emergence, plant height, plant dry weight, and % visual phytotoxicity of the treated plants are compared with an untreated control. Vegetative vigor is a 14-28 day foliar spray test conducted at one dose level, which compares the plant height, plant dry weight, and % visual phytotoxicity in the treated plants with an untreated control. The tests are usually conducted with crop species, such as corn, soybeans, and a root crop. Seven other species that may be used include tomato, cucumber, lettuce, cabbage, oat, ryegrass, and onion.
    - **Non-Target Area Aquatic Plant Phytotoxicity** is a laboratory test that evaluates the acute toxicity of fungicides at the highest application rate to a freshwater green alga (*Pseudokirchneria subcapitata*) and an aquatic macrophyte (*Lemna gibba*). For herbicides, five species are usually tested at the highest application rate: *Skeletonema costatum, Lemna gibba, Anabaena flos-aquae, Pseudokirchneria subcapitata*, and a freshwater diatom, usually *Navicula* sp.

- **Tier II**
    - **Non-Target Terrestrial Area Plant Phytotoxicity** is a greenhouse, growth chamber, or small plot test that consists of two parts: a seedling emergence test and a vegetative vigor test. These tests evaluate the effects of multiple dosage levels on plant growth. This test is used to generate an EC_{25} and NOAEC for % emergence, plant height, plant dry weight, and % visual phytotoxicity.
    - **Non-Target Aquatic Area Plant Phytotoxicity** is a dose- response test that is designed to evaluate the acute toxicity of pesticides to five aquatic species: *Pseudokirchneria subcapitata* (a freshwater green alga), *Lemna gibba* (an aquatic macrophyte), *Anabaena flos-aquae* (a blue-green alga), *Skeletonema costatum* (a marine diatom), and an unspecified freshwater diatom, usually *Navicula Pellicosa*. This test is used to generate EC_{50} and NOAEC values.
- **Tier III**
    - **Non-Target Plant Phytotoxicity Field Studies** are terrestrial and aquatic field tests that may be required on a case-by-case basis if terrestrial plants show greater than 25% adverse effects on plant growth and aquatic plants show greater than 50% adverse effects on plant growth. These tests provide critical information on harmful effects to plants during stages of development.
    - **Target Area Phytotoxicity Testing** provides data concerning the phytotoxic effects of a pesticide on desirable plants.

On June 27-29, 2001, EPA and the EXIT Pest Management Regulatory Agency of Health Canada, presented a harmonized four-tiered testing design for aquatic and terrestrial plants to the Scientific Advisory Panel. As part of this testing scheme, the two agencies proposed expanding the number of tested plant species to account for the wide variability in plant responses to chemicals. Each tier or level of progression in the testing scheme requires a more refined assessment of hazard and exposure. Presentations and results of this meeting are available at 2001 SAP Meetings.

How OPP Uses Ecotoxicity Data

After reviewing an individual toxicity or ecological effects study for a pesticide, EPA scientists develop a **data evaluation record** (DER) for the study. A DER summarizes the toxicity to certain species groups that are expected to be exposed to the pesticide. The templates for these DERs can be accessed at Environmental Effects Data Evaluation Record (DER) Templates.

The conclusions from all the individual ecotoxicity DERs are then integrated and summarized in a stressor-response profile, the final product of the ecological effects characterization. The profile presents the suite of effects for various animals and plants and an interpretation of available incidents information and monitoring data. Guidance for using incident data in evaluating animal and plant species (listed and non-listed) can be found at: Guidance for Using Incident Data in Evaluating Listed and Non-listed Species under Registration Review. The Agency compares the stressor-response profile with potential exposure levels to determine the risk of exposure-related effects.

In developing its ecological effects characterization, EPA uses either a five-step or a three-step scale of toxicity categories to classify pesticides based on toxicity data:

Ecotoxicity Categories for Terrestrial and Aquatic Organisms

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Toxicity Category	Avian: Acute Oral Concentration (mg/kg-bw)	Avian: Dietary Concentration (mg/kg-diet)	Aquatic Organisms: Acute Concentration (mg/L)	Wild Mammals: Acute Oral Concentration (mg/kg-bw)	Non-Tar Insects: Concent (µg/bee)
very highly toxic	<10	<50	<0.1	<10	
highly toxic	10-50	50-500	0.1 - 1	10 - 50	<2
moderately toxic	51-500	501-1000	>1 - 10	51 - 500	2 - 11
slightly toxic	501-2000	1001-5000	>10 - 100	501 - 2000	
practically nontoxic	>2000	>5000	>100	>2000	>11

Next Section: Analysis - Exposure Characterization

Previous Section: Problem Formulation

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