



## ENVIRONMENTAL PROTECTION AGENCY

[EPA-HQ-OPPT-2009-0154; FRL-9333-1]

### Final Test Guidelines; OCSPP 850 Series; Notice of Availability

**AGENCY:** Environmental Protection Agency (EPA).

**ACTION:** Notice.

**SUMMARY:** EPA is announcing the availability of the final test guidelines for Series 850--Ecological Effects Test Guidelines, consisting of Groups B, C, D, and F. These test guidelines are part of a series of test guidelines established by Office of Chemical Safety and Pollution Prevention (OCSPP) for use in testing pesticides and chemical substances to develop data for submission to the Agency under the Toxic Substances Control Act (TSCA), the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), and section 408 of the Federal Food, Drug and Cosmetic (FFDCA). As guidance documents, the test guidelines are not binding on either EPA or any outside parties.

**FOR FURTHER INFORMATION CONTACT:** *For general information contact:*

Melissa Chun, Regulatory Coordination Staff (7101M), Office of Chemical Safety and Pollution Prevention, Environmental Protection Agency, 1200 Pennsylvania Ave., NW., Washington, DC 20460-0001; telephone number: (202) 564-1605; email address: [chun.melissa@epa.gov](mailto:chun.melissa@epa.gov).

*For technical information contact:* Amy Blankinship, Environmental Fate and Effects Division (7507P), Office of Pesticide Programs, Environmental Protection Agency, 1200 Pennsylvania Ave., NW., Washington, DC 20460-0001; telephone number: (703) 347-8062; email address: [blankinship.amy@epa.gov](mailto:blankinship.amy@epa.gov), or Kathryn

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Gallagher, Risk Assessment Division (7403M), Office of Pollution Prevention and Toxics, Environmental Protection Agency, 1200 Pennsylvania Ave., NW., Washington, DC 20460-0001; telephone number: (202) 564-1398; email address: *gallagher.kathryn@epa.gov*.

## **SUPPLEMENTARY INFORMATION:**

### **I. Introduction**

These test guidelines are part of a series of test guidelines established by OCSPP for use in testing pesticides and chemical substances to develop data for submission to the Agency under TSCA (15 U.S.C. 2601 *et seq.*), FIFRA (7 U.S.C. 136 *et seq.*), and section 408 of FFDCA (21 U.S.C. 346a).

The test guidelines serve as a compendium of accepted scientific methodologies and protocols that are intended to provide data to inform regulatory decisions under TSCA, FIFRA, and/or FFDCA. The test guidelines provide guidance for conducting the test, and are also used by EPA, the public, and the companies that are subject to data submission requirements under TSCA, FIFRA, and/or FFDCA.

As guidance documents, the test guidelines are not binding on either EPA or any outside parties, and EPA may depart from the test guidelines where circumstances warrant and without prior notice. At places in this guidance, the Agency uses the word “should.” In this guidance, use of “should” with regard to an action means that the action is recommended rather than mandatory. The procedures contained in the test guidelines are recommended for generating the data that are the subject of the test guideline, but EPA recognizes that departures may be appropriate in specific situations. Alternatives to the recommendations described in the test guidelines may be proposed, and the Agency

will assess them for appropriateness on a case-by-case basis.

## **II. General Information**

### *A. Does this Action Apply to Me?*

This action is directed to the public in general. Although this action may be of particular interest to those persons who are or may be required to conduct testing of pesticides and chemical substances for submission to EPA under TSCA, FIFRA, and/or FFDCFA, the Agency has not attempted to describe all the specific entities that may be affected by this action. If you have any questions regarding the applicability of this action to a particular entity, consult the technical person listed under **FOR FURTHER INFORMATION CONTACT**.

### *B. How Can I Get Copies of this Document and Other Related Information?*

1. *Docket for this document.* The docket for this action, identified by docket identification (ID) number EPA-HQ-OPPT-2009-0154, is available at <http://www.regulations.gov> or at the Office of Pollution Prevention and Toxics Docket (OPPT Docket), Environmental Protection Agency Docket Center (EPA/DC), EPA West Bldg., Rm. 3334, 1301 Constitution Ave., NW., Washington, DC. The Public Reading Room is open from 8:30 a.m. to 4:30 p.m., Monday through Friday, excluding legal holidays. The telephone number for the Public Reading Room is (202) 566-1744, and the telephone number for the OPPT Docket is (202) 566-0280. Please review the visitor instructions and additional information about the docket available at <http://www.epa.gov/dockets>.

2. *Electronic access to OCSPP test guidelines.* To access OCSPP test guidelines electronically, please go to <http://www.epa.gov/ocspp/pubs/frs/home/testmeth.htm>. You may also access the test guidelines at <http://www.regulations.gov> grouped by series under docket ID numbers: EPA-HQ-OPPT-2009-0150 through EPA-HQ-OPPT-2009-0159 and EPA-HQ-OPPT-2009-0576.

### **III. Overview**

#### *A. What Action is EPA Taking?*

EPA is announcing the availability of final test guidelines under Series 850-- Ecological Effects Test Guidelines, consisting of Groups B, C, D, and F:

- Group B--Terrestrial Wildlife.
- Group C--Terrestrial Beneficial Insects, Invertebrates, and Soil and Wastewater Microorganisms.
- Group D--Terrestrial and Aquatic Plants, Cyanobacteria, and Terrestrial Soil Core Microcosm.
- Group F--Field Test Data Reporting Guidelines.

OCSPP, formerly the Office of Prevention, Pesticides and Toxic Substances (OPPTS), established a unified library of test guidelines for use in developing data for submission to EPA under TSCA, FFDCA, and/or FIFRA. Beginning in 1991, EPA initiated an effort to harmonize the test guidelines within OCSPP, as well as to harmonize the OCSPP test guidelines with those of the Organization for Economic Cooperation and Development (OECD). The process for developing and amending these test guidelines has included public participation and the extensive involvement of the scientific community, including peer review by the FIFRA Scientific Advisory Panel (SAP), the

Scientific Advisory Board (SAB), and other expert scientific organizations. With this notice, EPA is announcing the availability of the final test guidelines OCSPP 850 Series, consisting of Groups B, C, D, and F dealing with ecological effects for use in testing chemical substances and developing data for submission to EPA. Test guidelines in this series were made available for public comment by a notice document published in the **Federal Register** issue of March 4, 1996 (61 FR 8279) (FRL-4990-3). The peer review on May 29, 1996 by FIFRA SAP was announced in a meeting notice published in the **Federal Register** issue of May 1, 1996 (61 FR 19276) (FRL-5366-3). The test guidelines were subsequently revised in response to FIFRA SAP and public comments.

Based on comments from FIFRA SAP and from the public, the following changes were made in the final test guidelines:

1. *Group titles and organization changes.* EPA is changing the title of Group D “Nontarget Plants Test Guidelines” to “Terrestrial and Aquatic Plants, Cyanobacteria, and Terrestrial Soil Core Microcosm Test Guidelines.” The term “nontarget,” which is not applicable to OPPT, is removed from the Group D title, broadening the scope to all test guidelines evaluating effects to plants. The following test guidelines involving plants are renumbered and moved to Group D: OCSPP 850.4500 “Algal Toxicity” (Public Draft OPPTS 850.5400) and OCSPP 850.4900 “Terrestrial Soil-Core Microcosm Test” (Public Draft OPPTS 850.2450).

EPA is changing the title of Group C “Beneficial Insects and Invertebrates Test Guidelines” to “Terrestrial Beneficial Insects, Invertebrates, and Soil and Wastewater Microorganism Test Guidelines,” expanding the scope to include testing of microorganisms other than the aquatic algae. The following microorganism test

guidelines are renumbered and moved to Group C: OCSPP 850.3200 “Soil Microbial Community Toxicity Test” (Public Draft OPPTS 850.5100) and OCSPP 850.3300 “Modified Activated Sludge, Respiration Inhibition Test” (Public Draft OPPTS 850.6800).

EPA is moving the “Earthworm Subchronic Toxicity Test” test guideline from the Public Draft Group F “Chemical Specific Test Guidelines” to Group C and renumbering it from OPPTS 850.6200 to OCSPP 850.3100. The earthworm is being added to Group C since it is a beneficial soil invertebrate.

EPA is changing the Group G designation to Group F, and the test guideline contained within it (Public Draft OPPTS 850.7100) is renumbered OCSPP 850.6100. The title for Group E is removed as it no longer contains any test guidelines; however, Group E and its title are reserved.

*2. Title change for OCSPP 850.4000.* EPA is changing the title of OCSPP 850.4000 “Background--Nontarget Plant Testing” to “Background and Special Considerations: Tests with Terrestrial and Aquatic Plants, Cyanobacteria, and Terrestrial Soil-Core Microcosms.” The new title reflects the change in the Group D title.

*3. Background and special consideration test guideline addition for Group B and Group C and content revision of Group D.* EPA is adding two background and special consideration test guidelines: OCSPP 850.2000 “Background and Special Considerations: Terrestrial Wildlife” and OCSPP 850.3000 “Background and Special Considerations: Terrestrial Beneficial Insects, Invertebrates, and Soil and Wastewater Microorganisms.” The addition of these test guidelines are in response to comments regarding harmonizing the organization of test guidelines and improving the consistency of terminology and

guidance applicable across test guidelines in a group. These test guidelines provide general guidance on test methods, statistics, and data reporting and an overview of the use for OPPT and OPP. Such test guidelines already exist for Group A (OPPTS 850.1000) and Group D (OCSPP 850.4000). Information contained within the OCSPP 850.2000 and OCSPP 850.3000 is based on information extracted from the test guidelines within their respective group and on general statistical methods applicable to toxicity testing.

With the addition of test guidelines from other groups, OCSPP 850.4000 was updated to reflect general information applicable across test guidelines in Group D. This information was extracted from the existing test guidelines. Additionally, a description of the meaning of the terms “Tier I,” “Tier II,” and “Tier III,” under TSCA in contrast to their definitions under FIFRA, was added.

*4. Title changes and merging and splitting of test guidelines--i. Removal of terms “Tiers I, II, and III” from test guideline titles and consolidation of resulting common test guidelines.* The terms “Tier I,” “Tier II,” or “Tier III” used in these test guideline titles are not necessary and are misleading as they have different regulatory meanings under OPP and OPPT. These tests, though, are used by both programs. EPA is changing the OPPTS 850.4400 “Aquatic Plant Toxicity Test Using *Lemna* Spp., Tiers I and II” and OPPTS 850.5400 (now OCSPP 850.4500) “Algal Toxicity, Tiers I and II” test guideline titles by removing “Tiers I and II.”

EPA is also removing the terms “Tier I” and “Tier II” and then consolidating and harmonizing the “Tier I” test guidelines with their “Tier II” test guideline counterparts for the following test guidelines: The “Terrestrial Plant Toxicity, Tier I (Seedling

Emergence)” and “Seedling Emergence, Tier II” test guidelines (Public Drafts OPPTS 850.4100 and OPPTS 850.4225, respectively). These test guidelines were merged and harmonized into OCSPP 850.4100 “Seedling Emergence and Seedling Growth.” The “Terrestrial Plant Toxicity, Tier I (Vegetative Vigor)” and “Vegetative Vigor, Tier II” test guidelines (Public Draft OPPTS 850.4150 and OPPTS 850.4250, respectively) were merged and harmonized into OCSPP 850.4150 “Vegetative Vigor.” For these test guidelines, except for the number of treatment levels, “Tier I” test conditions (referred to as limit tests) are essentially the same as “Tier II” (definitive tests) test conditions.

EPA is changing the title of OPPTS 850.4450 “Aquatic Plants Field Study, Tier III” test guideline by removing the term, “Tier III.”

EPA is also removing the term “Tier III” from the OPPTS 850.4300 “Terrestrial Plants Field Study, Tier III” test guideline. Public Draft OPPTS 850.4025 “Target Area Phytotoxicity” was merged with Public Draft OPPTS 850.4300 to create a single test guideline, OCSPP 850.4300 “Terrestrial Plants Field Study.” The target area test guideline covers a special case of a terrestrial plant field study for OPP, where the study area is the area intentionally treated with a pesticide when label use directions are followed. The OCSPP 850.4300 “Terrestrial Plants Field Study” provides flexibility to cover this special case for OPP, if needed.

ii. *Division of the Algal Toxicity Test into two separate guidelines.* EPA split the Public Draft OPPTS 850.5400 “Algal Toxicity, Tiers I and II” test guideline into two test guidelines: OCSPP 850.4500 “Algal Toxicity” and OCSPP 850.4550 “Cyanobacteria (*Anabaena flos-aquae*) Toxicity” (in addition to removing “Tiers I and II”). This division of the unicellular species into two different test guidelines provides a clearer

differentiation between methodological approaches prescribed for testing cyanobacteria and those for testing the unicellular algae. Additionally, this division addresses the reclassification of blue-green algae as cyanobacteria.

*5. Standardization of test guideline organization.* The FIFRA SAP recommended that the ecological effects test guidelines include the same organizational format and that the tables summarizing test conditions for appropriate test guidelines contain consistent concepts across test guidelines. As a result of these suggestions, information was moved within the test guidelines, but the information remained the same. Tables summarizing test conditions and test validity elements were added to test guidelines in which species specific or laboratory measurements were defined. In all test guidelines where a calculated response measure (e.g., average specific growth rate) was derived from direct response measures (e.g., weight), equations were provided.

*6. Highlights of technical changes--i. Addition of a limit test option.* Public comments indicated that a limit test could be an option to a definitive test in additional test guidelines. A limit test provides an opportunity to reduce the number of animals to be tested and/or resources. Test guidelines where a limit test is appropriate and a limit test option was added include the following: OCSPP 850.2200 “Avian Dietary Toxicity Test;” OCSPP 850.2400 “Wild Mammal Toxicity Test;” OCSPP 850.3100 “Earthworm Subchronic Toxicity Test;” OCSPP 850.3300 “Modified Activated Sludge, Respiration Inhibition Test;” OCSPP 850.4230 “Early Seedling Growth Toxicity Test;” and OCSPP 850.4600 “Rhizobium-Legume Toxicity.” Although a limit test option is available for OCSPP 850.2100, OCSPP 850.2200, and OCSPP 850.2400, language was added that if sublethal effects are observed at the limit dose, a definitive test should be conducted.

ii. *Modification of limit dosage or concentration “cut-off” values.* The limit dosage or concentration values for tests for pesticides were originally set at values seen in the literature as “cut off” values. It was believed that few, if any, pesticides would be applied at a label rate that would result in residues equal to or greater than these values. Based on current exposure models used within the Office of Pesticide Programs (OPP), though, there are cases where estimated environmental residue values are higher than limit values provided in the public drafts, and there are also cases where actual or expected environmental exposure levels may be higher than the limit values for industrial chemicals. To address these case-by-case occurrences, language was added saying that the limit value should be adjusted upward if environmental exposure concentrations are expected to be higher than the limit value. In addition, guidance on how to calculate a pesticide estimated environmental concentration for comparison to a typical limit value was included in each test guideline with a limit test option.

iii. *Group B test guidelines.* In OCSPP 850.2100 “Avian Acute Oral Toxicity Test,” passerine species and alternative species were added as test species in response to FIFRA SAP comments for additional alternative test species and the new passerine 40 CFR part 158 data requirement published in the **Federal Register** issue of October 26, 2007 (72 FR 60934-60988) (FRL-8106-5). Furthermore, the option of testing additional sex and age groups (including breeding females) on a case-by-case basis, as well as confirmation of dosing solutions, were added to address comments. OCSPP 850.2200 “Avian Dietary Toxicity” was modified to specify that young birds cannot survive 5 days without feeding. Additionally, when delayed effects are observed or expected, the guidance extending the observation period recommends testing for at least 14 days but

continuing until overt evidence of toxicity has subsided.

There were also issues raised with the cage sizes provided in OCSPP 850.2300 “Avian Reproduction Test.” In response to these comments, the specific cage sizes provided in OCSPP 850.2300 were removed and replaced with a recommendation to follow current best practices for the care and testing of laboratory animals, as recommended cage sizes for avian species for use in reproductive tests are evolving. The health and presence/absence of signs of stress in control animals are used to help evaluate housing and handling conditions. Additionally, language was added to increase the minimum number of replicate pens per treatment to 16, and the measurement endpoint of hatchling body weight was added. Furthermore, the initial test subject age was reduced to as low as 16 weeks to address comments on problems of starting with older test subjects and impacts on acclimation, holding, and initial photoperiod during the reproduction phase.

iv. *Group C test guidelines.* In OCSPP 850.3020 “Honey Bee Acute Contact Toxicity” and OCSPP 850.3030 “Honey Bee Toxicity of Residues on Foliage,” the age of test bees was harmonized with that in OECD 214 “Honeybees, Acute Contact Toxicity Test” and with FIFRA SAP comments. A method for immobilizing bees using cold temperature was included and the wording on the source of bees was changed to an “apparently disease-free colony” in response to comments on collection techniques and source of bees. In addition, language regarding measurements of residue concentrations on the foliage was added.

v. *Group D test guidelines.* In conducting ecological risk assessments, both hypothesis-based endpoints (No Observed Effect Concentration/Lowest Observed Effect

Concentration (NOEC/LOEC)) and regression-based endpoints (e.g., inhibition concentration  $IC_{25}$  or  $IC_{50}$ ) are recommended in OCSPP 850.4100 “Seedling Emergence and Seedling Growth,” OCSPP 850.4150 “Vegetative Vigor,” OCSPP 850.4400 “Aquatic Plant Toxicity Test Using *Lemna* spp.,” OCSPP 850.4500 “Algal Toxicity,” and OCSPP 850.4550 “Cyanobacteria (*Anabaena flos-aquae*) Toxicity.” Within these test guidelines, text was modified to clarify both objectives and test acceptability in terms of both definitive and limit tests. A number of modifications to OCSPP 850.4400 were made to harmonize the test guideline with OECD 221 “*Lemna* sp Growth Inhibition.” A change in the period of testing from 14 days to 7 days was made in alignment with the OECD 221 guideline based on bridging data between 7-day and 14-day results. An evaluation of in-house toxicity data on *Lemna gibba* demonstrated no significant difference between the inhibition concentration endpoint values at 7 days versus 14 days.

The minimum number of replicates has been increased to 4 for OCSPP 850.4100 “Seedling Emergence and Seedling Growth,” OCSPP 850.4150 “Vegetative Vigor,” OCSPP 850.4400 “Aquatic Plant Toxicity Testing Using *Lemna* spp.,” OCSPP 850.4500 “Algal Toxicity,” and OCSPP 850.4550 “Cyanobacteria (*Anabaena flos-aquae*) Toxicity” to reflect the objective of these tests within OPP in which a hypothesis-based no observable adverse effect concentration (NOAEC) in addition to the regression-based  $IC_x$  is calculated. The NOAEC is used in endangered and threatened species assessments and there are cases where nonparametric tests, which require a minimum of 4 replicates, are needed to evaluate the results. Additional modifications to OCSPP 850.4550 were made to reflect FIFRA SAP recommendations of not continuously shaking test vessels during the test and using sonification only to facilitate counting.

*B. How Were These Test Guidelines Developed?*

OCSPP has developed a unified library of test guidelines, which are used in the testing of pesticides and toxic substances, and in the development of test data to meet the data requirements of the Agency or for voluntary testing purposes. Test guidelines are documents that specify methods that EPA recommends for generating data to support the registration of a pesticide, for setting of a tolerance or tolerance exemption for pesticide residues, or for the decisionmaking process for an industrial chemical. These test data are used by the Agency to perform risk assessments and make regulatory decisions. Studies conducted according to these test guidelines may be required under FIFRA (7 U.S.C. 136) for pesticide registration, pursuant to 40 CFR part 158. Test guideline studies may also be useful for satisfying FIFRA data requirements either in data call-ins issued pursuant to FIFRA section 3(c)(2)(B) or as needed to satisfy data requirements appropriate for specific pesticide registration applications, or for satisfying data requirements to demonstrate the safety of a tolerance or tolerance exemption under FFDCa section 408 (21 U.S.C. 346a).

Test guidelines used in regulatory actions as the basis for test standards under TSCA (15 U.S.C. 2601) are typically promulgated in 40 CFR part 799. They may also be written into specific TSCA rules such as TSCA section 4 test rules or consent orders or they may be used as recommended test guidelines as part of voluntary testing. Note that where data will be required under a TSCA rule, such as a test rule under TSCA section 4, a TSCA-specific version of the applicable test guideline may be promulgated as a rule. Examples of specific chemical test rules and consent orders may be found in 40 CFR part 799, subparts B and C.

The availability of public draft test guidelines for public comment was announced in a March 4, 1996 **Federal Register** notice. The public draft test guidelines were placed in the EPA Docket for public access. These public draft test guidelines were also submitted by EPA to FIFRA SAP on May 29, 1996, for peer review and was announced in a May 1, 1996 **Federal Register** notice. These final test guidelines incorporate changes recommended by FIFRA SAP and other changes resulting from the public comments received in response to the 1996 public draft test guidelines. The majority of comments and changes dealt with the organizational structure of the test guideline groups, consistency of organization and format across the ecological effects guidelines, addition of tables summarizing test conditions, addition of tables summarizing test validity elements, consistency in use of terminology, and updating of references. The reporting section of each test guideline now provides a list of study specific information to include in a study report based on study reporting requirements specified in 40 CFR 160.185 for FIFRA and 40 CFR 792.185 for TSCA.

**List of Subjects**

Environmental protection, Chemical testing, Test guideline.

Dated: June 19, 2012.

James Jones,

*Acting Assistant Administrator, Office of Chemical Safety and Pollution Prevention.*

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