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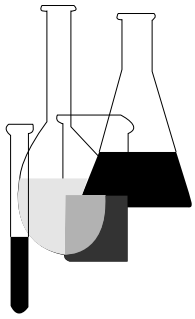
Biochemicals Test Guidelines

OPPTS 880.3800
Immune Response



INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

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OPPTS 880.3800 Immune response.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*).

(2) **Background.** The source material used in developing this harmonized OPPTS test guideline is OPP guideline 152–24.

(b) **Immunotoxicity studies with biochemical pest control agents (BPCAs): tier II—When required.** Data on alterations of immune responses (tier II) are required by 40 CFR 158.165 to support the registration of each manufacturing-use and each end-use product when significant immunotoxic effects are observed in test animals treated in any of the tier I immunotoxicity studies (OPPTS 880.3550), or when the immunotoxicity data from the tier I studies cannot be definitively interpreted, or when there is available data from other sources which indicate that the test substance, or structurally-related substances (including metabolites and degradation products) are immunotoxic.

(c) **Test standards**—(1) **Principles of the test methods.** The tests and methods are designed to:

(i) Provide information on the time-course of recovery from each significant adverse immunotoxic effect observed in tier I studies.

(ii) Indicate whether the observed effects may result in impaired host resistance to infectious microbial agents and/or to tumor cell challenge.

(iii) To provide additional information essential for a full evaluation of potential risks associated with the immunotoxicityof a test substance.

(2) **Test selection.** (i) Selection of the appropriate tests to be done in tier II studies will depend on:

(A) The particular test or tests in tier I in which significant immunotoxic effects were observed.

(B) The availability of data from other sources which indicate that the test substance, or structurally-related substances, are immunotoxic.

(C) Whether data from initial testing in tier II indicate that expanded testing is required, using additional tests in this tier.

(ii) Consultation with appropriate representatives of the Agency is suggested to determine which tests are to be performed.

(d) **Immunotoxicity studies**—(1) **Recovery from adverse immunotoxic effects.** For each study in tier I (OPPTS 880.3550) where significant immunotoxic effects were observed (including histopathological effects), the satellite group of treated test animals (OPPTS 880.3550(c)(3)) is to be used to evaluate the time-course for recovery from the effects

after withdrawal of the test substance. The tests should be conducted in accordance with the protocols set forth for each test in OPPTS 880.3550, with the following modifications:

(i) At least five treated animals in the satellite group and five untreated control animals are to be evaluated at 7, 14, and 28 days after the withdrawal of the test substance.

(ii) Only those tests in OPPTS 880.3550 in which significant immunotoxic effects were observed need be repeated with the satellite group of test animals.

(iii) If a satellite group of test animals, or control animals for the satellite group, were not included as suggested in the tier I studies, or if these animals were insufficient in number for proper evaluation of recovery from any adverse immunotoxic effects observed in the tier I studies, then the necessary tests should be initiated as described in OPPTS 880.3550, and completed as described above with the appropriate numbers of test and control animals.

(iv) Studies in tier I may be required, in which both sexes of several strains of test animals are exposed to the BPCA at several dose levels for longer time periods (e.g., 90 days).

(2) **Altered host resistance after challenge with infectious agents or with tumor cells.** (i) The selection of an appropriate host resistance model (or models) will depend largely on the particular immunotoxic effects observed from testing at the tier I level (OPPTS 880.3550) and, on whether the model system has been established as providing reproducible data for detecting altered host resistance after exposure to environmental chemicals.

(ii) Host resistance models that may be appropriate for use when alterations of cell-mediated immune responses are observed in tier I tests include challenge with Listeria monocytogenes or challenge with transplantable syngeneic (or semi-syngeneic) tumor cells, including PYB6 fibrosarcoma or B16F10 melanoma tumor of C57BL/6 mice; or challenge with Herpes simplex virus type 1 or type 2 (HSV-1, HSV-2).

(iii) Host resistance models that may be appropriate for use when alterations of humoral-mediated immune responses are observed in tier I tests include challenge with Streptococcus sp. (e.g., Streptococcus pyogenes) or challenge with HSV-1 or HSV-2.

(iv) Other models, not mentioned above, involving challenge with other infectious agents or tumor cells, also may prove useful in evaluating host resistance to BPCAs.

(v) It may be considered appropriate to include host resistance models as substitutes for certain immunotoxicity tests recommended in tier I

(OPPTS 880.3550). However, documentation must be provided to show that the immune system parameters measured by the selected host resistance models are equivalent to those evaluated in the tests suggested in tier I.

(vi) General test standards. (A) The resistance of host animals to challenge by infectious agents or tumor cells usually will involve exposing young adult rodents for 30 days to the highest dose of BPCA that did not lead to overt toxicity in the test animals.

(B) Reporting on parameters, appropriate for each host resistance model system, other than the endpoint of mortality, should be considered so that proper interpretation can be made of any observed changes in host resistance.

(C) Appropriate positive control groups of test animals, dosed with a known immunosuppressive chemical, should be included with each host resistance system used.

(D) In addition to providing all relevant data appropriate for each host resistance system used, protocols should be sufficiently well-described, and justification/ reasoning should be provided for any modifications in protocols of standard techniques.

(E) Current knowledge on the immune system components involved in host resistance to the infectious agent or tumor cell should be included in the report.

(F) In-depth host resistance studies may be required, in which several strains and both sexes of host animal (weanling to aged) are exposed to the BPCA at several dose levels for longer time periods (e.g., 90 days).

(3) **Other immunotoxicological studies.** (i) Additional tests may be required if considered necessary for a full evaluation of potential risks associated with the immunotoxicity of a BPCA. These include, but are not necessarily limited to, available tests that are designed to evaluate effects of a substance on:

(A) Lymphoid organs and tissues (using enzyme and immune histochemistry).

(B) Serum complement.

(C) Antibody response to T-independent antigens.

(D) Enumeration of subpopulations of T- and B- lymphocytes.

(E) Granulocyte function.

(F) Bone marrow function.

(G) Lymphokines.

(H) Plaque-forming cell response to T-independent antigens.

(I) Mitog stimulation of lymphocyte blastogenesis.

(J) In vivo popliteal lymph node enlargement after injection of allogeneic lymphocytes.

(K) Hormones.

(L) Immune system development.

(M) Macrophage development, activation and function.

(N) Induction of autoimmunity.

(ii) Certain additional tests may be required if evidence is obtained which indicates that the test substance is immunogenic.

(iii) When required. Tests to measure these and other parameters of the immune system may be required when indicated by:

(A) The extent and/or nature of the immunotoxic effects observed in the tier I (OPPTS 880.3550) tests.

(B) The nature of immunotoxic effects observed in the host resistance challenge models.

(C) The inability to definitively interpret the data from tier I studies or from the host resistance challenge models;

(D) The necessity to conform, by using alternate tests, the results from tier I studies.

(E) The availability of data from other sources which indicate that the test substance, or closely related substances (including metabolites and degradation products) are immunotoxic.

(e) **References.** The following references should be consulted for additional background material on this test guideline. The following are publications that either provide useful protocols for the design of immunotoxicity studies, or contain citations for useful protocols.

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