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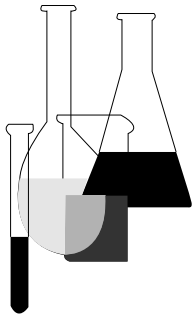
Microbial Pesticide Test Guidelines

OPPTS 885.0001 Overview for Microbial Pest Control Agents



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 885.0001 Overview for microbial pest control agents (MPCA).

(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 are OPP guidelines 150A, 152A–31, 152A–32, and 152A–33.

(b) **General.** (1) This series provides testing and informational guidelines for data to be submitted to support registration of MPCAs. The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) establishes the Agency's authority over the distribution and use of pesticide products. Before the Agency can register a pesticide, FIFRA requires the Agency to have sufficient data to determine that the pesticide, when used in accordance with widespread and commonly recognized practice, will not cause (or significantly increase the risk of) unreasonable adverse effects to humans or the environment (section 3(c)(5) and (7) of FIFRA).

(2) The Code of Federal Regulations at 40 CFR part 158 specifies the kinds of data and information that must be submitted to EPA to support the registration of each pesticide. This series of guidelines (OPPTS 885) provides detailed information relating to the data requirements listed in § 158.740, including the conditions under which each data requirement is applicable; the standards for acceptable testing; the information that should be included in a test report; guidance on evaluation and reporting of data; and examples of protocols. In addition, scientific publications are cited in the guidelines to provide useful information for designing test protocols.

(c) **Discussion of microbial pesticides test guidelines.** (1) Biological and biologically derived pesticides are typically naturally occurring, specific to the target species, and typically have unique or nontoxic modes of action. Because of these factors, biological pesticides are most appropriately characterized for health and environmental safety by testing schemes which take their unique characteristics into account. Pesticides referred to as MPCAs include (but are not limited to) bacteria, algae, fungi, viruses, and protozoa as defined in 40 CFR 152.20. The guidelines apply to all MPCAs used as pesticides, including both those that are naturally occurring, and those that are strain-improved, either by natural selection or by deliberate genetic manipulation.

(2) These guidelines were developed for MPCAs in order to address the special testing needs for these products. Unlike chemical pesticides, MPCAs may survive and reproduce in the environment, and may infect or cause disease in other living organisms. Thus, the basic testing protocols are designed specifically to detect any of these characteristics. Protocols

for further testing emphasize exposure or environmental expression in addition to expanded testing of infectivity and pathogenicity.

(d) **Background of microbial pesticides test guidelines**—(1) **History.** (i) The first microbial pesticide (*Bacillus popilliae*, a naturally occurring bacterium) was registered in 1948. During the late 1960s and early 1970s, interest in microbial pesticides began to increase. These products are registered for use in agriculture, forestry, mosquito control, and homeowner situations.

(ii) In 1974, in recognition of the growing interest in, and concern about, microbial pesticides, EPA began to sponsor a variety of workshops, symposia, and panel discussions aimed at identifying the relevant safety concerns for microbial pesticides. As early as 1978, at an EPA symposium titled ''Viral Pesticides: Present Knowledge and Potential Effect on Public and Environmental Health,'' the need for sensitive identification and detection methods for microorganisms as well as quality assurance provisions were clearly identified.

(iii) The Office of Pesticide Programs (OPP) issued a Policy Statement on Biorational Pesticides. In it, OPP recognized microbial and biochemical pesticides as distinct from conventional chemical pesticides, and made the commitment to develop appropriate testing guidelines. In 1979, OPP commissioned an American Institute of Biological Sciences' expert panel to develop a ''Human Hazard Evaluation Scheme for Biorational Pesticides.'' The final report of this expert panel formed the basis for the mammalian toxicology unit of the testing guidelines for microbial pesticides.

(iv) OPP issued testing guidelines for microbial and biochemical pesticides as Subdivision M of the Pesticide Assessment Guidelines (published through the National Technical Information Service (NTIS) in 1983 (EPA–540/9–82–028)). The microbial pesticide portion of the Subdivision M guidelines applies to both naturally occurring and genetically modified pesticides. It was decided at that time that any additional data that would be required for the registration of genetically modified microorganisms would be determined on a case-by-case basis by EPA.

(v) **Revisions to Subdivision M.** The Agency has gained considerable experience in the risk assessment of MPCAs since Subdivision M was published in 1983. Accordingly, the need for revising and updating portions of the guidelines became apparent. After review by the FIFRA Scientific Advisory Panel, a revision intended to better address the needs of both testing laboratories and scientific staff was published in l989 and reflects an extensive updating of testing guidelines for MPCAs. The revised guidelines utilize the tier testing scheme set forth in 1983 with the original publication of Subdivision M to ensure, to the greatest extent possible, that only the minimum data sufficient to make scientifically sound regu-

latory decisions will be required. The Agency expects most of MPCAs will require testing only in the first tier. Moverover, the Agency believes that the Tier I test requirements represent a reasonable approach to evaluating risk related to the use of pesticides, and is one in which negative results would allow a high degree of confidence in the safety of the test agents.

(e) **Discussion of registration, field testing, and experimental use permits**—(1) **Registration.** Data requirements for pesticide registration appear at § 158.740.

(i) MPCA testing is required in the areas of product analysis, toxicology, residue analyses on food crops, and ecological effects and environmental expression. Revised data requirements are listed under paragraph (i) of this guideline.

(ii) Product analysis requirements (OPPTS Series 885, Group A) include the necessary data and information to identify the active ingredient and any inert substances that have been added, and to guard against chemical and biological contamination both prior to registration and during production of the MPCA. This information is required for all MPCAS.

(iii) Toxicology requirements (OPPTS Series 885, Group C) are set forth in three tiers. Tier I consists of a battery of short term tests designed to evaluate potential for toxicity, infectivity, and pathogenicity. Tier II is designed to evaluate the particular situation when, in the absence of evidence of pathogenicity, either toxicity or infectivity is observed in Tier I. Tier III contains tests that may resolve issues of known or suspected human pathogenicity and tests for particular adverse effects of intracellular parasites of mammalian cells.

(iv) Residue data (OPPTS Series 885, Group B) describe the quantity of MPCA or its associated toxins that might appear on food or feed crops. These data are required only if there are significant human health concerns arising from the toxicology testing.

(v) Ecological effects and environmental expression testing (OPPTS Series 885, Group D and E) also have been grouped in tiers. Tier I, consists of maximum dose single species hazard testing on nontarget organisms. If adverse effects are observed in Tier I, the potential exposure to the MPCA is estimated by means of Tier II testing for population dynamics, (fate and expression) in the environment. If Tier II tests show that there may be significant exposure to the MPCA, Tier Ill studies to determine a dose response effect or to examine certain chronic effects will be performed to determine if the minimum infective dose is less than the exposure or if there are other considerations that would decrease the observed effects in the environment. Tier IV tests, under simulated or actual environmental conditions, are to be designed on a case-by-case basis to evaluate any specific problem that cannot be resolved by lower tier testing.

(2) **Small scale field testing**—(i) **Background.** The provisions for obtaining experimental use permits (EUP) for testing pesticides are codified in 40 CFR part 172. This regulation includes a presumption that an EUP will not be necessary for small-scale field testing (nonfood uses on not more than 10 acres of land or not more than 1 surface-acre of water, providing that the water not be used for irrigation purposes, drinking water supplies, or body-contact recreational activities). Such a presumption is appropriate for chemical pesticides which have no independent mobility or reproductive capability and, therefore, when applied in small-scale field studies generally have very limited potential for causing adverse effects outside the treated area. Similarly, when used in small-scale field tests, naturally-occurring microbial pesticides are subject to natural control or dissipation mechanisms.

(ii) **Small scale field testing of certain genetically altered microbial pesticides.** Genetically altered microbial pesticides may not be subject to natural control or dissipation mechanisms and thus may be capable of spreading beyond the site of application with the potential for causing adverse effects. Therefore, small-scale field studies with these types of microbial pesticides could raise many of the same concerns as more extensive use of conventional pesticides, and the presumption that an EUP is not required may not be appropriate for these pesticides. To address these issues, the Agency developed an interim policy requiring notification under FIFRA prior to small-scale field testing of genetically altered and non-indigenous microbial pesticides in order to determine the need for EUPs prior to release of these MPCAs into the environment. In 1994, the Agency amended 40 CFR Part 172 to provide for notification, with data required, prior to small-scale field testing of genetically modified organisms.

(iii) **Large scale field testing and EUPs.** (A) The Agency encourages applicants for EUPs to consult with OPP scientists to develop an appropriate testing scheme. The data required prior to field testing MPCAs may consist of any, or all, of the tests required for registration, since these living microorganisms have the potential to multiply to high exposure levels, depending on their ability to survive, reproduce, and compete for dominance in the environment. However, the Agency recognizes the need to limit testing in the course of development of pesticides and intends to make every effort to restrict field testing data requirements to only those necessary to evaluate that particular field test.

(B) Reduced data requirements for EUPs may be justified on a case-by-case basis by consideration of certain exposure factors (e.g., limited capacity for the MPCA to survive at, or disseminate from, the field test site, and containment or mitigation provisions in the test protocols). Human health and ecological effects testing will be limited to the most likely areas of concern as predicted by a careful consideration of the known properties of the MPCA and similar microorganisms. As much information as possible should be submitted to the Agency to allow for anal-

ysis of the potential risks of the field tests. Specific information recommendations for this preliminary assessment of both notifications and EUP applications are discussed in paragraph (f) of this guideline.

(3) **Principles for justifying data waivers.** (i) The full battery of tests for registration of MPCAs was designed to give basic hazard and exposure information for a microorganism with totally unknown properties. In actual practice, an MPCA is usually well identified, which may facilitate prediction of its properties and behavior. This is particularly true for the areas of human health and plant pathogenicity. Clinical medicine and agricultural science have identified most microorganisms associated with diseases. If an MPCA is taxonomically similar to a clinically or agriculturally significant microorganism, this particular area of concern should be examined closely, possibly by requiring additional testing (as provided in 40 CFR 158.75) beyond that specified in 40 CFR 158.740. Conversely, if the MPCA belongs to a group of microorganisms that have never been found in association with any disease, a case may be made for reducing, or waiving, the testing requirements for this area of concern.

(ii) 40 CFR part 158 contains provisions for granting waivers for data requirements in response to specific written requests by applicants (40 CFR 158.45). OPP encourages applicants to discuss their preliminary testing plans with OPP scientists. Waivers of some testing requirements may be appropriate for certain MPCAs. This tailoring of the testing battery on a case-by-case basis relies on both an accurate description of the MPCA and the existence of a reliable taxonomy for the class of microorganism to which it belongs. Some microorganisms have been more closely examined than others and have a larger data base from which to draw conclusions. In addition, certain kinds of microorganisms are more amenable to classification than others. In general, human and plant pathogenic bacteria have been best classified due to their health and economic significance. Other microorganisms, particularly protozoa and fungi, might not be as well studied or described, and it may be difficult to predict their properties reliably from a taxonomic description. In this case, it may be more difficult to justify waiving test requirements.

(iii) An additional factor in determining the extent of testing that may be necessary for risk assessment is the degree of species specificity shown by the MPCA. This is of primary importance in assessing ecological risk. Most MPCAs are designed to produce adverse effects against a target species. Careful scientific consideration on a case-by-case basis must be given to the selection of nontarget species to be tested (e.g., beneficial insects, environmentally or commercially significant plants, or wildlife) in order to include species that are most likely to be susceptible.

(iv) Because of the difficulty in providing definitive exposure predictions from currently available ecological methods, the Agency takes the approach that hazard testing on nontarget organisms should be submitted

initially (Tier I tests). If significant adverse effects are identified in Tier I tests, ecological exposure tests (Tier II) are performed to attempt to quantify levels of the' MTCA to which the susceptible nontarget species may be exposed. Although normally requested at a Tier 11 level, definitive ecological exposure data showing that the MPCA will not survive or persist in the environment would be good support for a request for waiver of some or all of Tier I testing requirements.

(f) **Definitions.** The following definitions apply to this series of guidelines:

Animal means all vertebrate and invertebrate species, including, but not limited to, humans and other mammals, birds, fish, and shellfish.

Aquatic animals means all vertebrates and invertebrates that inhabit fresh, estuarine, or marine waters for all or part of their life cycles.

Aquatic use means the use of a pesticide in a fresh water, estuarine, or marine aquatic system by either direct application or direct discharge of treated water.

Biological control agent means a living organism introduced into the environment to control the population or biological activities of another life form considered to be a pest under section 2(t) of FIFRA.

Dose means a quantity of material, whether living or not, to be applied to an animal at one time.

Dosing regimen means a systematic schedule of doses.

End-use product (EP) is an MPCA containing product that is registered or intended for direct use or application for pest control purposes. In some cases, an EP is identical to the manufacturinguse product (the technical grade of the active ingredient or formulation intermediate (Fl)). In other cases, an EP is formulated from the manufacturing use product by addition of inert ingredients such as antioxidants or other stabilizers, suspending agents, carriers, encapsulating materials, wetting agents, or anticaking compounds. The intentionally added inerts may influence MPCA storage stability/viability as well as deposition and persistence at the end use site. In some cases, an EP is manufactured via an integrated formulation process, i.e. the manufacturing product used for formulation is not a separate registered product.

Environmental expression means the extent and manner in which a microorganism establishes and maintains its presence in an ecological habitat.

Estimated environmental concentration means an estimate of the concentration of an MPCA occurring in or on various media (i.e., soil, water,

air) after pesticide application, as determined from the results of environmental fate or expression Tier II testing.

ID50 means the amount of material required to produce overt disease symptoms in 50 percent of the test animals.

Infectivity is the ability of a microorganism to cross or evade natural host barriers to infection.

Maximum expected environmental concentration means the highest concentration of a pesticide occurring at any given time (usually immediately after application) at a site or in a medium (e.g., water, vegetation, or soil) as determined from the pesticide application rate.

Manufacturing use product (MP) is a preparation containing the MPCA in question that is used to formulate an EP. An MP either is the technical grade of the active ingredient or a formulation ingredient (FI) (a product containing the technical grade of the active ingredient to which other ingredients have been added deliberately (e.g. stabilizers, dispersants, diluents)).

Maximum hazard testing means a testing scheme that is designed to maximize any toxic or pathogenic effects of the test substance on the test (nontarget) organism.

Microbial pest control agent means any of those microorganisms including (but not limited to) bacteria, fungi, viruses, and protozoa as defined in 40 CFR 162 that are used to control pests.

Morbidity means the evident state of disease.

Moribund means approaching death.

Mortality means the state of an animal or plant in which all vital functions have ceased.

Natural occurrence means the presence of an organism in its normal habitat where it grows, develops, and reproduces.

Pathogenicity is the ability to inflict injury and damage in the host after infection, and depends on host resistance or susceptibility.

Plant means any member of the plant kingdom.

Pure active form of each ingredient (PAI) is a preparation containing pesticidal functioning units of the MPCA in question obtained after the application of the most rigorous purification procedures. Where techniques are used to alter the MPCA genetically, the genetically altered strain is considered as the basis for defining the PAI of the MPCA. The purest form of the MPCA that can be obtained is a preparation that is free of any other biological forms and free of contandnating growth media or host

substrate material. Chemical pesticidal product from genes that have been engineered into a microorganism also may be considered as separate active ingredients.

Purest infective form (PIF) means that preparation of infective virus containing the least amount of extraneous material.

Technical grade of the active ingredient (TGAI) is a material containing the MPCA in question which is produced commercially, or m a manner equivalent to the planned commercial process, and to which no ingredient has been added intentionally except for purposes of MPCA growth or replication, or typical purification. The TGAI is considered to be the purest preparation resulting from a typical production process, and is the preparation intended for distribution and/or formulation into a formulation intermediate (FI) or end-use product (EP). Where techniques are used to alter the MPCA genetically, the genetically altered strain is considered as the basis for defining the technical grade of the MPCA. Each pesticidal product from genes that have been engineered into a microorganism also is considered as an active ingredient. The technical grade of each pesticidal product from introduced genes is that form which exists along with the technical grade of the MPCA in question after a typical production process.

Terrestrial wildlife means nondomestic birds or animals.

Toxicity is the injury or damage in a host caused by a poison or toxin where infection by and/or replication or viability of the microorganism are not necessarily required.

Toxin means a poisonous substance, generated by a microorganism, plant, or animal, capable of causing injury or damage when it interacts with host cells.

Typical end-use product means a pesticide product representative of a major formulation category (e.g. emulsifiable concentrate, granular product, wettable powder) that contains the active ingredient of a registration applicant’s product.

Virulence factors means the traits of a microorganism that allow for pathogenicity.

(g) **Basic standards for testing.** The standards contained in this guideline apply to all studies in this series of guidelines unless specifically modified for use in a specific method.

(1) **Test substance for biological and environmental studies.** It is advised that appropriate representatives of the EPA be consiulted prior to testing in order to determine the form/purity of the MPCA that should be tested to support the registration of each MP and each EP. It is recognized that certain forms of the MPCA may be inappropriate for certain

tests. In general, the form (e.g. vegetative cell, spore, cyst, virion) of the microorganism to be tested should be equivalent to the form that is intended for registration. The test microorganism also should be equivalent to that intended for registration with respect to stage of growth, possession of organelles and appendages, and expression of phenotypic traits (including products from genes that have been intentionally introduced into the microorganism). If significant exposure to other forms of the microorganism is expected, or if changes in form of the microorganism occur, or are expected to occur in target,or nontarget species, these forms may have to be tested also. In general, the following principles should be followed:

(i) Tests requiring use of the TGAI shall be conducted with the manufacturing-use product if the TGAI and MP are identical, or with the TGAI used to produce the manufacturing-use or end-use formulated pesticide product if not identical.

(ii) The lot of the substance tested should be the same throughout the duration of the study, and the test sample should be stored under conditions that maintain purity and stability. If the stability of the test substance cannot be maintained for the duration of the study or if, for other reasons, it is not possible to use the same lot throughout the test, subsequent lots of the test substance shall be selected to be as nearly identical to the original lot as practical. Chemical or biological assays shall be performed to ensure composition identity and consistency.

(iii) Each lot of the test substance shall be analyzed, to the limits of technical feasibility, and the name and quantities of ingredients, contaminants, and impurities listed. The determination shall include the quantity of unknown material, if any, so that 100 percent of the test sample is accounted for. The test substance shall be within the limits of purity, if any, certified in accordance with OPPTS 885.1500.

(iv) If the test or control substance is to be incorporated into feed or other vehicle, the period during which the test or control substance is stable or viable in such a mixture should be determined prior to the start of the study. No mixture of test or control substance with the feed or vehicle shall be maintained or used during a period exceeding the known stability or viability of the test or control substance in the mixture. Alternatively, determinations of the stability or viability of the test or control substance in random samples of the diet or vehicle mixture shall be made at least monthly during the study to ensure that proper mixing, formulation, and storage procedures are being followed and that the appropriate concentration of the test or control substance is contained in the mixture.

(v) If the test or control substance is incorporated into feed or other vehicle, its homogeneity and concentration in the diet shall be determined prior to the start of the study and, each time a new mixture is prepared. Random samples of the mixture shall be analyzed at least monthly to en-

sure that proper mixing, formulation, and storage procedures are being followed, and that the appropriate concentration of the test or control substance is contained in the mixture.

(vi) In addition to or in lieu of data otherwise required by this guideline, the Agency may require, after consultation with the applicant, data derived from testing to be conducted with:

(A) An analytically or microbiologically (e.g., (PIF) for viruses) pure grade of an active ingredient.

(B) The labile form of infectious material (e.g. nonoccluded virus).

(C) An inert ingredient of a pesticide formulation.

(D) A contaminant or impurity.

(E) A metabolite (from animals or plants) or degradation product of an active or inert ingredient.

(F) The end-use formulated product.

(G) Any additional substance (including other pesticides recommended for tank mixing with the test substance) that enhances the virulence or toxicity of the product for which registration is sought.

(H) Any combination of the substances mentioned above.

(2) **Administration or application of test substance and vehicles.** (i) The manner of administration or application of the test and control substance for biological or environmental testing shall be selected to maintain accuracy of the dosage or treatment.

(ii) A vehicle other than water or saline used to dissolve or dilute the test substance or positive control substance shall be chosen to possess the following characteristics if possible:

(A) It does not alter the absorption, distribution, metabolism, or retention of the test substance.

(B) It does not alter the chemical or biological properties of the test substance or enhance, reduce, or alter the pathogenic or toxic characteristics of the test substance.

(C) At the levels used in the study, it does not produce physiological effects and is nontoxic.

(D) It should be identical to, or closely resemble the vehicle, if any, used in the pesticide product. It should be identical to the vehicle if possible.

(3) **Controls for biological and environmental studies**—(i) **Need for controls.** Controls are used in biological or environmental studies required by the guidelines in OPPTS Series 885 to ensure that observed effects are associated with the test substance exposure. The appropriate control groups shall be identical in every respect to the treated groups except for exposure to the test substance. In studies involving animals or plants, all controls shall, to the extent possible, be from the same source, be of the same age, receive the same care, and receive the same nutrients as the animals or plants receiving the test substance. To prevent bias, a method to assign organisms to treatment and control groups randomly is required and must be referenced in the report.

(ii) **Untreated (negative) controls.** Untreated (negative) control groups are usually required. Untreated controls receive neither the test substance nor any ancillary material (vehicle).

(iii) **Controls treated with inactivated MPCAs.** In certain circumstances, deleterious effects may be produced in test animals through a mechanism other than active infection (e.g. anaphylaxis). This control group may provide information useful in determining the mechanism of pathogenesis.

(iv) **Vehicle control groups.** (A) If a vehicle other than water or saline is used to administer the test substance, a concurrent vehicle control group may be required. Vehicle control groups receive treatment with the vehicle alone, and the vehicle is usually administered at the highest level that the vehicle is administered in any test group in the study. Consult individual sections of this guideline for those tests where a vehicle control is required or recommended.

(B) As provided in paragraph (h)(3)(iii)(A) of this guideline, the vehicle should be selected on the basis of information establishing that it is nontoxic at the levels used in the study, has no independent physiological effects, and does not alter the chemistry, pathogenicity, or toxicity of the test substance. If, however, there are insufficient data on the effects of the vehicle, testing of the vehicle is required.

(v) **Positive controls.** Positive controls generally are not required. These serve as internal quality controls, and demonstrate known test organism sensitivity and respond to known toxic or infective agents. They are also used to ascertain if a strain or species reacts similarly to another strain or species when exposed to the same known or standard toxicant or infective agent. Consult individual sections of this guideline for those tests where a positive control is required or recommended.

(vi) **Historical controls.** Historical control data are required when the Agency desires information on longevity,, spontaneous diseases, or other characteristics of a species or strain selected for study, and for certain

comparative or statistical purposes. Consult individual guidelines for those tests where historical control data are required.

(vii) **Additional controls.** Additional controls may be required as dictated by test design.

(h) **Special test requirements.** In addition to the data required in this series of guidelines, data derived from other tests may, under unusual circumstances, be required by the Agency in order to make judgments regarding safety to humans, domestic animals, and other nontarget organisms. Such data will be required where special problems are encountered. Test methods will usually be derived from tests already described or cited in other guidelines of this series. Such data requests may relate to a proposed pattern of use, a toxicological mode of action, or a unique chemical or microbial property. The data requested will be specific to the problem. Examples of test requirements for unusual circumstances include but are not limited to: Certain chemical property data from OPPTS Series 830 (Product Properties Test Guidelines) and certain toxicity data from OPPTS Series 870 ((Health Effects Test Guidelines).

(i) **Reporting of data.** Each test report submitted under this series of guidelines shall satisfy the reporting requirements of this paragraph, unless specific instructions direct otherwise. Data should be submitted to the Agency in hard copy format. In addition, whenever possible, copies should be submitted in machine readable form by computer disk or via direct electronic lines.

(1) **General requirements**—(i) **Identification.** Each test shall identify:

(A) The name and address of the laboratory or site where the test was performed.

(B) The party or parties primarily responsible for any written or other matter contained in the report, and the portions of the report for which each party is responsible.

(ii) **Verification.** Each test report shall be:

(A) Signed by each of the senior scientific personnel, including the laboratory director, responsible for performing and supervising the testing and preparing, reviewing, and approving the test report.

(B) Certified by the applicant or an authorized agent of the applicant as a complete and unaltered copy of the report provided by the testing laboratory, whether independent or owned, operated, or controlled by the applicant.

(2) **Format and content.** The test report shall include all information necessary to provide a complete and accurate description and evaluation

of the test procedures and results. The test report shall contain at least four parts: A summary and evaluation of the test results, a description of the test procedures, a listing of the data and information required by each applicable section of this guideline, and a section in which data and findings are discussed. Metric units of measurement must be used although English units may be included where appropriate. The systems may not be mixed (e.g. milligrains per quart).

(3) **Summary of test results.** This section of the test report is to contain a summary of the data and significant findings.

(4) **Description of the test procedure.** This section of the test report is to contain a full description of the test procedure. If applicants believe any of the reporting requirements are not applicable, they must submit an explanatory statement to this effect. A full description of the test procedure should include but not be limited to:

(i) **Deviation from standards.** The report must indicate all ways in which the test procedure fails to meet applicable standards for acceptable testing contained in this guideline, and must state the reasons for such deviations.

(ii) **Test methods.** Specification of test methods, including a full description of the experimental design and procedures, and the length of the study (including the dates on which the study began and ended) is to be stated.

(iii) **Substance tested.** Identification of the test substance is to be provided, including:

(A) If the test substance is microbiological: Scientific name and, to the extent possible, serotype and strain or other appropriate designated type, and, to the extent possible, a qualitative and quantitative determination of composition (including names and quantities of known contaminants and impurities, within technically feasible limits). The determination shall also include quantities of unknown materials, if any, to account for 100 percent of the sample.

(B) Manufacturer and lot number of the test substance, and relevant properties of the substance tested, (i.e. physical state, pH, and purity).

(C) Identification and composition of any vehicles or other materials (e.g. diluents, suspending agents, emulsifiers, virulence enhancers) used in administering the test substance.

(iv) **Animal and plant data.** Animal and plant data should include:

(A) Species and strain used and reasons for selection of species (if the species is other than the species reconunended or required by the Agency).

(B) Source of supply of test organisms.

(C) Disease history of the test animals.

(D) Description of any pretest conditioning.

(E) Method used in randomly assigning animals or plants to test or control groups.

(F) Numbers of animals of efach sex in each test or control group.

(G) Age and condition of animals or plants at beginning of study.

(v) **Environmental conditions.** A description of the environmental conditions under which the testing was conducted is to be reported. Further details may be provided by specific testing methods.

(vi) **Treatment or doses.** For studies where test substance applications, treatments, or dosings are made, a complete description of such is to be reported. Further details may be provided by specific testing sections.

(vii) **Treatment for diseases not caused by the test substance.** Test animals or plants with a history of disease are not be used for microbial pesticide testing. The feed must be antibiotic free. For MPCAs where test organisms have been treated with some agent or manipulated by some system to prevent or control infectious diseases not caused by the test substance, a full description of such treatment or manipulation must be reported. Such description should include:

(A) Identification of the test organisms affected and the disease organism involved.

(B) The nature and severity of the disease.

(C) The date of onset and duration of the disease.

(D) The nature of the treatment or manipulation used to control or eliminate the disease, and the dates of such actions.

(E) The outcome of the treatments in relation to the disease and to the test results.

(viii) **Observations.** Method, frequency, and duration of observations made during the study are to be reported. Other related specific information to be reported may be provided by specific testing methods.

(ix) **Availability of raw data, specimens, and samples of the test substances.** The location of all raw data, specimens and samples of the test substances which are retained in accordance with 40 CFR, part 160 and OPPTS 885.1200, and the name and address of the individual responsible for the archives and the name and address of the recognized culture collection, must be reported.

(x) **References.** References must be provided for the statistical and other methods employed for analyzing the data, and for any published literature used in developing the test protocol, performing the testing, making and interpreting the observations, and compiling and evaluating the results.

(xi) **Reporting the results and evaluation of specific tests.** The test results and any evaluations of test results should be reported in accordance with the requirements of the individual specific testing sections of these guidelines. Such results and evaluations include all data, information, and analysis necessary to support the registration application and its corresponding product label claims, directions, and precautions. The report must be sufficiently detailed that a reviewing scientist has sufficient information to reach an independent conclusion from the data.

(xii) **Discussion section.** The discussion section of the test report must contain a full scientific discussion of any and all positive or unexpected negative results and findings. All aberrant data must be noted and explanations based on sound scientific principles must be presented. Any conclusions arrived at by the study authors should be included.

(5) **Statistical procedures**—(i) **General.** Appropriate statistical methods are to be used to summarized experimental data, to express trends, ad to evaluate the significance of differences in data obtained from different test groups. The methods used shall reflect the current state-of-the-art.

(ii) **Standard deviation and standard error.** All data averages or means must be accompanied by standard deviations, to indicate the amount of variability in the data. In addition, the standard errors of the means should also be calculated, to compare means from, different test groups; however, notations of statistically significant differences accompanied by the confidence level or probability should also be used in place of standard error determinations. Other methods of expressing data dispersion may also be used when appropriate.

(j) **EUP data requirements for MPCAs**—(1) **Overview.** (i) Data to support applications for EUPs for microbial pesticides generally include those data that would ordinarily be generated during the initial stages of product development. For example, most product analysis information would be developed early in the product development stages, and the Tier I toxicology and nontarget organism toxicity tests would usually be conducted first in preparation for registration. Unless these test results indicate toxic, pathogenic, or other harmful properties, no data on residues or environmental fate would ordinarily be necessary.

(ii) As indicated in paragraph (d)(3) of this guideline, the Agency recognizes the need to limit testing expenses in the development of microbial pesticides and will make every effort to grant data waiver requests

where justified. Anyone planning to submit an EUP should consult with Agency scientists in order to develop an appropriate testing scheme.

(iii) In accordance with 40 CFR 172.3, no EUP will be required for nonfood-use tests conducted on a cumulative total of not more than 10 acres of land or not more than 1 surface acre of water providing that the water is not used for irrigation, drinking water, or body contact recreational activities, or that the water not contain or affect any fish shellfish or other plants or animals used for food or feed. The Agency requires notification prior to conducting small-scale field tests of certain genetically altered MPCAs.

(2) **General provisions.** The data required for an EUP are denoted by square brackets (e.g. [C]) in tables in 40 CFR 158.740. When requesting preliminary assistance from Agency scientists in determining a data testing scheme, as much of the following information on the MPCA as possible should be available. This kind of information will be used to determine the specific tests needed or to determine the appropriateness of approving test waiver requests.

(i) The identity of the MPCA including:

(A) Characteristics.

(B) Means and limit of detection.

(ii) Description of its natural habitat including information on:

(A) Predators.

(B) Parasites.

(C) Competitors.

(iii) Information on the host range, with an assessment of infectivity and pathogenicity to nontarget organisms.

(iv) Information on the population dynamics of the microorganism in the environment.

(v) A description of the proposed testing program including:

(A) The purpose or objectives of the proposed testing.

(B) Designation of the pest organisms involved.

(C) The States in which the proposed program will be conducted.

(D) The specific identity of the exact location of the test sites (including proximity to residences and human activites, surface water, etc.)

(E) The crops, fauna, flora, geographical description of sites, modes, dosage rates, frequency, and situation of application on or in which the pesticide is to be used.

(F) The amount of pesticide product proposed for use.

(G) The method of application.

(H) A comparison of the natural habitat of the microorganism with the proposed test site.

(I) The number of acres, structural sites, or animals/plants by state, to be treated or included in the area of experimental use.

(J) Procedures to be used to protect the test area from intrusion by unauthorized individuals.

(K) The proposed dates or periods during which the testing program is to be conducted, and the manner in which supervision of the program will be carried out.

(L) Description of procedures for monitoring the microorganism within and adjacent to the test site during the test.

(M) The method of disposal or sanitation of plants, animals, soils, etc., that were exposed during and after the field test.

(N) Means of evaluating potential adverse effects and methods of controlling the microorganism if detected beyond the test area.

(vi) A statement of composition for the formulation to be tested, giving:

(A) The name and percentage by weight of each ingredient, active and inert.

(B) Production methods.

(C) Extraneous microorganisms present as contaminants.

(D) Amount and potency of any toxin present.

(E) The number of viable microorganisms per unit weight or volume of the product (or other appropriate system for designating the quantity of active ingredient).

(vii) The following information applies to genetically altered MPCAs:

(A) Description of the methods used to alter the microorganism genetically.

(B) The identity and location of the rearranged or inserted/deleted gene segments in question (host source, nature, base sequence data, or restriction enzyme map of the genes).

(C) Information on the control region of the genes, and a description of the new traits or characteristics that are expressed.

(D) Data on the potential for genetic transfer and exchange with other organisms and on genetic stability of any inserted sequence.

(E) Data on the relative environmental competitiveness compared to the parental strains.