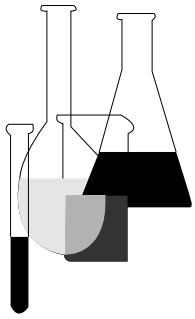
United States
Environmental Protection
Agency

Prevention, Pesticides
and Toxic Substances
(7101)

EPA 712–C–96–304
February 1996

Microbial Pesticide Test Guidelines

OPPTS 885.2350 Analytical Methods—Animals



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.*).

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on *The Federal Bulletin Board*. By modem dial 202–512–1387, telnet and ftp: fedbbs.access.gpo.gov (IP 162.140.64.19), or call 202–512–1530 for disks or paper copies. This guideline is also available electronically in ASCII and PDF (portable document format) from the EPA Public Access Gopher (gopher.epa.gov) under the heading ‘‘Environmental Test Methods and Guidelines.’’

OPPTS 885.2350 Analytical methods—animals.

(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 the OPP guideline 153A–8a.

(b) **Analytical methods.** Analytical methods are required both for data collection to support proposed tolerances and for the enforcement of such regulations (40 CFR 180). Note that a monitoring method is required for the determination of all microbial pest control agents (MPCAs) that are exempt from the requirements of tolerance. The Agency must have the monitoring method available in times of need and cannot afford the potentially long method development period in the event adverse effects are observed subsequent to registration. The methods must not be subject to interference due to substrate, reagents, or residues (cells, virions, toxin, etc.) of related or unrelated microbial agents whether naturally occurring and unregistered or whether an MPCA. A confirmatory procedure is also required for each residue of concern for both data collection and tolerance enforcement.

(1) **Description of method.** Each method must be fully described, or a reprint must be provided as well, as any necessary modifications. Each method must be validated by submitting recovery data and analyses of untreated control samples of representative animal commodities. The estimated sensitivity/detection limit must be provided for each tested commodity. Attempts must be made to determine if residues in or on treated commodities (aged or weathered) are extracted with the same efficiency as those from spiked samples used for recovery experiments.

(2) **Choice of analytical method.** Widely differing analytical methods may be required to identify and quantify all residues of toxicological concern derived from a given MPCA. If a biologically active microbial product is of concern, the more conventional analytical procedures such as gas chromatography, mass spectrometry, or high-pressure liquid chromatography are typically used. If the MPCA per se, a mutant, or a viable recipient of MPCA genetic material is a residue of toxicological concern, various immunological methods (such as enzyme-linked immunosorbent assay, dot-immunoassay) or molecular probe methods (such as dot hybridization procedure, Southern hybridization procedure, or restriction endonuclease mapping) may be used for identification and/or quantification. Since the above procedures do not necessarily determine viable MPCAs, culturing of tissues (maceration followed by dilution plating) or infectivity assays will frequently be necessary. Culturing or bioassays are also important as means of detecting MPCAs at levels below the detection limits of the above methods and, theoretically, as few as one viable mi-

crobe can be detected using enrichment techniques, if necessary. In some cases, microscopy may be useful.

(3) **Purity of MPCAs.** It may be possible to purify some viral MPCAs to the point of crystallization whereas other MPCAs may not be isolatable from host cells or host membranes in a viable form. Some MPCA residues may be bound (actively or passively) to cellular structures/components and their release must be attempted using procedures such as sonication, use of detergents, or hydrolytic steps (enzymatic, acid, or alkaline). Care must be taken to determine background levels of cross-reacting MPCAs since antigenic similarities and/or nucleic acid homology may exist in indigenous microbes to a greater or lesser extent. In some cases, care must also be taken to detect different viable forms of the MPCA in question (such as spores vs. vegetative cells, encapsulated vs. nonencapsulated, or yeast vs. mycelial forms) since antigenic determinants may be different or may be masked. In the case of genetically altered MPCAs, the methods must be specific enough to determine the MPCA in the presence of the parent, unmodified, indigenous strain of the same microbe which, generally, will differ only in a relatively small portion of nucleic acid (chromosomal or extrachromosomal).

(4) **Limitations on methods.** The regulatory methods must be relatively simple, rapid, specific, and sensitive and should not require blank samples, exotic equipment or reagents, or use of internal or procedural standards. If the Agency finds the regulatory methods adequate, they will be published or referenced in the FDA Pesticide Analytical Manual after an exemption from tolerance or a permanent tolerance has been established.