

Chapter 1

Biopesticide Oversight and Registration at the U.S. Environmental Protection Agency

John Leahy, Mike Mendelsohn,* John Kough, Russell Jones, and Nicole Berckes

Environmental Protection Agency, Biopesticides and Pollution Prevention Division (7511P), 1200 Pennsylvania Avenue, Washington, DC 20460

***E-mail: mendelsohn mike@epa.gov.**

The Environmental Protection Agency (EPA) is committed to encouraging the development and use of biopesticides and considers them inherently reduced-risk pesticides. Biopesticides (microbial pesticides, biochemical pesticides, and plant-incorporated protectants) are required to be evaluated by EPA. The Agency must make findings of “no unreasonable adverse effects” to man and the environment to support its registration decision to permit sale and distribution under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), as well as a “reasonable certainty of no harm” under the Federal Food, Drug, and Cosmetic Act (FFDCA) to permit residues in food and/or feed. This chapter will review areas including how EPA views the benefits of biopesticides, related laws and legal requirements, biopesticide registration, and biopesticide data requirements. EPA’s commitment to low risk biological pesticides as alternatives to conventional chemical pesticides will also be emphasized.

What are Biopesticides?

Biopesticides, also known as biological pesticides, are pesticides derived from natural materials such as animals, plants, bacteria, and certain minerals. Typically, biopesticides have unique modes of action and are considered reduced risk pesticides. Biopesticides fall into three major classes:

- Biochemical pesticides;
- Microbial pesticides; and
- Plant-incorporated protectants.

Biochemical Pesticides

Biochemical pesticides are naturally occurring substances or are synthetically derived equivalents that have a non-toxic mode of action to the target pest(s), and have a history of exposure to humans and the environment demonstrating minimal toxicity. Synthetically derived biochemical pesticides are equivalent to a naturally occurring chemical with such a history. Biochemical pesticides include, but are not limited to: semiochemicals (insect pheromones and kairomones), natural plant and insect regulators, naturally occurring repellents and attractants, induced resistance promoters, and enzymes. Biochemical pesticides typically degrade rapidly and are not persistent in the environment.

Biochemical pesticides, with the exception of pheromones, tend to have much less species-specificity and are broader spectrum pesticides than the microbials. They also may have lethal effects upon the target pest. Lethal but non-toxic biochemical pesticides include suffocating agents (e.g., soybean oil), dessicants (e.g., acetic acid), and abrasives (e.g., diatomaceous earth).

Microbial Pesticides

Microbial pesticides are microorganisms that produce a pesticidal effect. They have pesticidal modes of action that often include competition or inhibition, toxicity and even use of the target pest as a growth substrate. They may be:

- Eukaryotic microorganisms including, but not limited to, protozoa, algae, and fungi;
- Prokaryotic microorganisms, including, but not limited to, bacteria;
- Autonomous replicating microscopic elements, including, but not limited to, viruses.

Microbial pesticides can control many different kinds of pests, although each separate active ingredient is relatively specific for its target pest(s). For example, there are fungi that control certain weeds and other fungi that kill specific insects.

The most widely used microbial pesticides are subspecies and strains of *Bacillus thuringiensis*, or *Bt*. Each strain of this bacterium produces a different mix of proteins, and specifically kills one or a few related species of insect larvae. While some *Bt* strains control moth larvae feeding on plants, others are specific for larvae of flies and mosquitoes. The target insect species are determined by whether the particular *Bt* produces a protein that can bind to a larval gut receptor, thereby causing the insect larvae to starve.

Plant-Incorporated-Protectants (PIPs)

Consistent with the Coordinated Framework for Regulation of Biotechnology issued by the U.S. Office of Science and Technology Policy in 1986 (51 FR 23302) genetically modified (GM) crops with pesticidal traits fall under the oversight of EPA, the U.S. Department of Agriculture, and the U.S. Food and Drug Administration. EPA's oversight focuses on the pesticidal substance produced (e.g., *Bt Cry* proteins) and the genetic material necessary for its production in the plant (e.g., *Cry* genes). EPA calls this unique class of biotechnology-based pesticides plant-incorporated protectants (PIPs).

PIPs are pesticidal substances that plants produce and the genetic material that has been added to the plant. For example, scientists can take the gene for the *Bt* pesticidal protein and introduce the gene into the plant's own genetic material. Then the plant, instead of the *Bt* bacterium, manufactures the substance that destroys the pest. EPA regulates the protein and its genetic material, but not the plant itself.

How EPA Views Benefits of Biopesticides

In 1994, the Biopesticides and Pollution Prevention Division (BPPD) was established in EPA's Office of Pesticide Programs (OPP) to facilitate the registration of biopesticides. BPPD promotes the use of safer pesticides, including biopesticides, as components of integrated pest management (IPM) programs.

EPA is committed to encouraging the development and use of low risk biological pesticides as alternatives to conventional chemical pesticides (1). The Agency recognizes that these pesticides are often different in their mode of action and has employed numerous measures to facilitate the application process. These include distinct data requirements for microbial and biochemical biopesticides, consolidation of biological pesticide application processing to a single group within OPP, and regulatory relief activities (2). EPA is committed to the efficient, effective approval of safer pesticides as well as a transparent, predictable process in decision making.

Since biopesticides tend to pose fewer risks than conventional pesticides, EPA generally requires much less data to register a biopesticide than to register a conventional pesticide, and EPA's review times are shorter for biopesticides.

While biopesticides require less data and are registered in less time than conventional pesticides, EPA always conducts rigorous reviews to ensure that pesticides will not cause unreasonable adverse effects on human health or the environment. For EPA to be sure that a pesticide is safe, the Agency requires that registrants submit a variety of data about the composition, toxicity, degradation, and other characteristics of the pesticide. These data requirements are described in more detail later in this paper.

There are several benefits to using biopesticides, including:

- Decreased risk without affecting yield. Biopesticides—when used as a component of an IPM program—can greatly decrease the use of conventional pesticides, without affecting crop yield.

- Often less toxic. Generally, biopesticides are inherently less toxic than conventional pesticides and are safer to those using them.
- Often effective in very small quantities and decompose quickly. This can result in lower exposures and avoid pesticide pollution problems.
- Targeting of specific pests. Biopesticides generally affect only the target pest and closely related organisms, in contrast to broad spectrum, conventional pesticides that may affect non-target organisms such as birds, insects, and mammals.
- When used in rotation with conventional products, biopesticides can help prevent development of pest resistance problems.
- Improved residue management. Buyers and consumers are becoming increasingly selective in their purchasing habits. Illegal pesticide residues left on produce can result in loss of markets, fines, and other consumer avoidance. Biopesticides often contain natural products that are normally consumed and do not have residue concerns.

Many microbial and biochemical biopesticides are not intended to function as "stand-alone" pest control products to completely replace conventional pesticides. Instead, these biopesticides are most effective when used as a component of an IPM program because they generally affect only the target pest and closely related organisms.

Additionally, for agricultural use products, biopesticides typically qualify for a reduced restricted entry interval and have no pre-harvest interval. Restricted entry intervals are requirements that limit the time that workers can return to a field once it has been treated with a pesticide. Restricted entry intervals can delay or obstruct time-sensitive cultural practices. Many biopesticides also do not have harvest restrictions. A harvest restriction is a waiting period between when a pesticide is applied and when the treated crop can be harvested and marketed. The waiting period after treatment can often be several days. Biopesticides without harvest restrictions give a grower much greater flexibility during harvest.

Microbial and biochemical biopesticides are generally labeled for use on a wide range of crops. As a result, for some minor crops or obscure pest problems, a biopesticide may be available when no conventional product is registered for the use. In addition, for larger crops such as corn, soybean, and cotton, PIP biopesticides have reduced the use of more toxic conventional insecticides.

Overview of OPP and BPPD's Role

EPA's Office of Pesticide Programs (OPP), along with the Office of Chemical Safety and Pollution Prevention (OCSPP), works with 10 Regional Offices and other EPA program offices on a wide range of pesticide issues and topics, such as:

- Evaluating potential new pesticides and uses;
- Providing for Special Local Needs and emergency situations;
- Reviewing safety of older pesticides;
- Registering pesticide producing establishments;

- Enforcing pesticide requirements; and
- Pesticide field programs, such as the frontline implementation activities carried out by states, tribes, and EPA Regional pesticide experts.

OPP is comprised of nine divisions, three of which are divisions responsible for the registration of pesticides. The Biopesticides and Pollution Prevention Division (BPPD) is responsible for all regulatory activities associated with biologically-based pesticides. Within BPPD, the Biochemical Pesticides Branch and Microbial Pesticides branch are responsible for registering biochemical and microbial pesticides, respectively. Additionally, the Microbial Pesticides Branch registers PIPs and other biotechnology-related products.

BPPD also is working to reduce pesticide risk by promoting Integrated Pest Management (IPM) initiatives and coordinating the Pesticide Environmental Stewardship Program (PESP). BPPD's vision is to be a world leader in biopesticide regulation and pollution prevention. The mission of BPPD is to protect human health and the environment by reducing the risks of pesticides through registering biopesticides and through encouraging pollution prevention practices.

Main Statutes and Legal Requirements

EPA regulates the use of pesticides under the authority of two federal statutes: the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetic Act (FFDCA) (3)(4). Additionally, the Food Quality Protection Act of 1996 (FQPA) amended FIFRA and FFDCA setting tougher safety standards for new and old pesticides and to make uniform requirements regarding processed and unprocessed foods (5). Finally, the Pesticide Registration Improvement Act (PRIA) establishes pesticide registration service fees for registration actions in the three registering divisions of EPA's Office of Pesticide Programs (6).

Other statutes that play roles in the regulation of biopesticides include:

- Endangered Species Act;
- Migratory Bird Treaty Act; and
- Clean Water Act

The following descriptions give brief overviews of the main statutes, though such descriptions are not intended to be comprehensive.

FIFRA

FIFRA provides the basis for regulation, sale, distribution and use of pesticides in the U.S. FIFRA authorizes EPA to review and register pesticides for specified uses. EPA also has the authority to suspend or cancel the registration of a pesticide if subsequent information shows that continued use would pose unreasonable risks. Some key elements of FIFRA include:

- Is a product licensing statute; pesticide products must obtain an EPA registration before manufacture, transport, and sale
- Registration based on a risk/benefit standard
- Strong authority to require data--authority to issue Data Call-ins
- Ability to regulate pesticide use through labeling, packaging, composition, and disposal
- Emergency exemption authority--permits approval of unregistered uses of registered products on a time limited basis
- Ability to suspend or cancel a product's registration: appeals process, adjudicatory functions, etc.

Microbial, biochemical, and plant-incorporated protectant biopesticides are considered pesticides under FIFRA, and generally are required to be evaluated and registered by EPA under Section 3 of FIFRA. EPA must make a finding of no unreasonable adverse effects to man and the environment from use of the pesticide in order to support its registration decision.

FFDCA and FQPA

The Federal Food, Drug, and Cosmetic Act (FFDCA) authorizes EPA to set maximum residue levels, or tolerances, for pesticides used in or on foods or animal feed. Under FFDCA and amendments to both FFDCA and FIFRA under the FQPA, EPA must make a similar finding of a reasonable certainty of no harm if the use of such agents results in residues in food or feed. If the submitted information supports this safety finding, EPA may establish a numerical tolerance or an exemption from the requirement of a tolerance regarding those residues. As of this writing, no microbial pesticides or plant-incorporated protectants registered for food use have been required to obtain a numerical tolerance. Rather, exemptions from the requirement of a tolerance have been granted based on the finding of no significant adverse effects in the supporting data.

PRIA

In 2004, Congress passed the Pesticide Registration Improvement Act (PRIA) and established a registration fee-for-service system with specific fees and decision times by type of action. PRIA 3 is the second five-year extension of the original Act and was the result of support and collaboration from a coalition of industry, grower, environmental groups, and farm worker advocates. As biopesticides are usually inherently less toxic than conventional pesticides, biopesticide registrations require a significantly reduced data set compared to conventional registrations. Additionally, biopesticides can follow truncated decision review timelines as well as reduced registration fees.

Experimental Use Permits, Emergency Exemptions, and State and Local Need Registrations

In the process of pesticide development, field testing is often necessary to evaluate the efficacy of a pesticide. Title 40 CFR Part 172 describes when it is necessary to obtain an Experimental Use Permit (EUP) under Section 5 of FIFRA for testing unregistered pesticides. Briefly, the size of the outdoor test acreage is greater than a cumulative 10 acres of land or 1 surface acre of water, an EUP is required. Any food or feed crops involved in or affected by the tests must be destroyed or consumed only by experimental animals unless a tolerance or exemption from a tolerance has been established. These acreage limitations are applicable only for outdoor terrestrial and aquatic uses. For those pesticides being tested on sites for which acreage is not relevant (e.g., tree stumps, rodent control, structural treatments or bird repellents), the determination of the need for an EUP is made on a case-by-case basis.

Other criteria to determine when an EUP must be obtained are set forth in 40 CFR Part 172.3. An EUP is of limited duration and requires that the test be carried out under controlled conditions. For small-scale field tests of genetically modified microbial pesticides or non-indigenous microbial pesticides that USDA has not previously acted upon, applicants must submit a notification to EPA for determination of whether an experimental use permit is necessary, even if the testing is on less than 10 acres.

In addition to registration under Section 3 of FIFRA, there are two additional means under FIFRA whereby a pesticide product may be distributed in the absence of a Section 3 registration or an experimental use permit. One is pursuant to an emergency exemption under Section 18 of FIFRA. Under this section, Federal or State agencies may request limited approval for an unregistered use of a currently registered pesticide product or the use of an unregistered pesticide product. Such a request can only be granted when there is a potentially severe economic or human health impact and no other alternatives are available for pest control. A Section 18 exemption usually allows use of the particular pesticide product for a year; however, the duration of the exemption may be limited or expanded depending on the situation (7).

Cases also exist where a particular pesticide product may be registered for one or more uses, but not for a particular use which is determined by the State as being a special local need. In these cases, the State may register that use or formulation needed for the special local need under Section 24(c) of FIFRA provided that appropriate tolerances or exemptions from tolerance exist if food or feed uses are involved. The EPA has 90 days to disapprove of such State registrations.

Biopesticide Registration

Before a pesticide can be marketed and used in the United States, FIFRA requires that EPA evaluate the proposed pesticide to assure that its use will not pose unreasonable risks of harm to human health and the environment, including non-target species. This involves an extensive review of health and safety information.

Pesticide registration is also the process through which EPA examines the ingredients of a pesticide; the site or crop on which it is to be used; the amount, frequency, and timing of its use; and storage and disposal instructions. A pesticide cannot legally be used, sold, or distributed if it has not been registered with EPA's Office of Pesticide Programs. FIFRA Section 2 (u), defines the term "pesticide" as:

- (1) any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest;
- (2) any substance or mixture of substances intended for use as a plant regulator, defoliant, or desiccant; and
- (3) any nitrogen stabilizer.

EPA makes online resources, such as the Pesticide Registration Manual (also known as the Blue Book), available to assist applicants through the registration process (8).

As biopesticides are usually inherently less toxic than conventional pesticides, biopesticide registrations may require a significantly reduced data set compared to conventional registrations. Additionally, there are reduced associated timelines and fees to help expedite registration processes. Timeframes to register pesticide products vary dependent on the PRIA code assigned to the submission. Based on PRIA 3 decision review timelines and fees for FY 14/15, biopesticide submissions can range from 7 months and \$6,079 USD for a new non-food use (PRIA 3 code: B650) to 19 months and \$48,621 USD for a new food use active ingredient with a petition to establish a tolerance (PRIA 3 code: B580). This is compared to 12 months and \$12,156 USD for a conventional new non-food indoor use (PRIA 3 code: R260) and 24 months and over \$590,000 USD for a new food use active ingredient (PRIA 3 code: R010).

Additionally, the Agency recommends that registrants request a pre-submission meeting with the appropriate registering branch. The pre-submission meeting is an excellent opportunity to discuss products in development and steps to take to ensure a timely registration decision. All information exchanged at these meetings is held confidential until a pesticide registration submission is made.

Pheromone Regulatory Relief

The Agency acknowledges that use of certain types of pheromone products presents lower risk than conventional pesticides, and also acknowledges the unique properties of these niche-type products regarding their inherently narrow host range (9). To promote the use of pheromone products, the Agency initiated a regulatory relief program that allows flexible confidential statements of formula for pheromone experimental use permits (EUPs) to allow for active ingredient adjustments during the course of experimentation. The Agency has also published generic tolerances and relaxed the acreage cut-off when an EUP is required for pheromones.

EPA established the following exemptions from the requirement of a tolerance as a result of the pheromone regulatory relief program: 1) for inert materials in polymeric matrix dispensers (40 CFR 180.1122); 2) for pheromones in retrievably-sized polymeric matrix dispensers (40 CFR 180.1124); 3) for straight-chained lepidopteran pheromones (sprayables) (40 CFR 180.1153); and 4) for inert polymers in sprayable formulations, (40 CFR 180.1162). EPA further set forth certain policies raising the acreage limit to 250 acres for experimental use permit requirements for the testing of pheromones in polymeric matrix dispensers (59 FR 3681), for testing of non-food use broadcast pheromones (59 FR 34182), and for straight-chained pheromones (sprayables) (60 FR 168).

Products Exempt from Registration

EPA has determined that pest control organisms such as insect predators, nematodes, and macroscopic parasites are exempt from the requirements of FIFRA (40 CFR 152.20(a)). In addition, pheromones (and identical or substantially similar compounds) labeled for use only in pheromone traps for monitoring and pheromone traps in which those chemicals are the sole active ingredients are not subject to regulation under FIFRA (40 CFR 152.25(b)). However, the use of pheromones in traps in conjunction with conventional pesticides, in other application methods (other than traps), or for purposes other than monitoring, is subject to regulation under FIFRA.

Minimum risk pesticides that meet certain criteria are a special class of pesticides that are not subject to federal registration requirements because their ingredients, both active and inert, are demonstrably safe for the intended use. They are exempt from federal registration under section 25(b) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). EPA does not review or register pesticides that satisfy the 25(b) criteria (40 CFR 152.25(f)), though registration of these products is required by most states.

International Partnerships, Involvement, and Outreach

To streamline agency resources and promote international biopesticide registration, EPA and Health Canada's Pest Management Regulatory Agency (PMRA) have established a process for the joint review of biopesticide products. The procedure entails a joint pre-submission consultation to establish specific data requirements.

Joint reviews increase the efficiency of the registration process, facilitate simultaneous registration in Canada and the U.S., and increase access to new pest management tools in both countries. Efficient work-sharing requires a mutual understanding of the responsibilities of each agency, as well as common procedures and time frames (10).

EPA has been an active member of the Organisation for Economic Co-operation and Development's (OECD) Biopesticide Steering Group (BPSG) which meets annually to discuss harmonization of guidelines and principles of

risk assessment. Comparisons and modifications of guidelines for toxicity and pathogenicity studies are vetted within the BPSG to reach consensus on risk assessment procedures for a variety of microorganisms used in pest management. In addition, specific organisms are reviewed to ensure that the latest scientific information on their biology is considered when evaluating their safe use in pest management. Production of toxins or secondary metabolites by some microbial pest control agents (MPCA) are of concern and it is critical that risk managers understand the prevalence of these compounds in products intended for environmental release.

While the BPSG brings together a broad range of scientists from many countries, not all aspects of dossier formatting, concerns over aspects of study guidelines and which studies are critical for risk assessment will be agreed upon by all members. Despite this, the BPSG provides an important forum for discussion on a wide range of topics and is the only such venue to reach such a broad range of MPCA developers and regulators. The greater the degree of harmonization of data requirements among member countries resulting from these interactions, the more likely reduced-risk biopesticides will find widespread use in agriculture.

Regarding international outreach, EPA's Office of Pesticide Programs meets periodically with representatives from several countries to discuss products of biotechnology and their impact on trade of agricultural commodities. Updates on regulatory approvals and assessment of novel traits are presented to U.S. and foreign governmental representatives for consideration and discussion. Asynchronous approval of biotechnology products by trading partners has led to occasional rejections of shipments of commodities at great expense and disruption of trade. These meetings provide a forum for direct interaction between regulators and a greater understanding of the risk assessment process as the U.S. is often seen as the lead country in the development and regulation of genetically engineered crops. The ultimate goal of these exchanges is the acceptance of risk management decisions (i.e., approvals) from one country by an importing country without the need for a separate additional review process.

Biopesticide Data Requirements

Looking at the data that is required for biopesticide registration, biochemical and microbial pesticides are subject to a different set of data requirements for registration than conventional chemicals. These Data Requirements for Registration, which are tiered, are listed in 40 CFR Part 158: Subpart U Biochemical Pesticides 158.2000 and Subpart V: Microbial Pesticides 158.2100. EPA has published guidance for developing these data in the Biochemical Pesticides Test Guidelines, OSCPP Series 880 and the Microbial Pesticides Test Guidelines, OSCPP Series 885.

The current regulations allow for flexibility in fulfilling the required data. This can be accomplished through providing a rationale as to why a specific test is not practical to perform, or by providing scientific rationale to address the particular

endpoint. In addition, the Agency has the authority to invoke additional testing requirements if a potential risk has been identified and needs to be investigated. This flexible approach ensures that potential risks presented by biopesticides will be properly assessed.

Biochemical Data Requirements

Product Analysis and Mammalian Toxicology

In general, the product characterization information required for biochemical pesticides is the same as required for conventional chemical pesticides. These include:

- Data/information on product identity and composition;
- Information on manufacturing process; and
- Discussion of the formation of impurities, enforcement analytical methods, analysis for certification of limits, and physical/chemical properties.

The Agency has adopted a tiered testing scheme to assure the safety of biochemical pest control agents toward mammalian species, similar to that used for microbial pesticides, and is comprised of three tiers. Adverse effects in a lower tier will trigger additional testing in the next higher tier (11) (12).

The mammalian toxicology studies generally required for registration in or on a terrestrial food crop include, in Tier I, acute toxicity tests (oral, dermal, and inhalation exposures, & primary dermal and primary eye irritation studies).

In addition, a battery of genotoxicity studies, 90- day oral, dermal, and inhalation studies (depending upon likely routes of repeated exposure), an immunotoxicity study, and a developmental toxicity study may be required. Hypersensitivity incidents are to be reported, if they occur. The Agency has, on a case by case basis, considered scientifically valid information or peer reviewed literature in lieu of guideline studies. In many cases, lack of significant exposure serves as a basis for not requiring active ingredient or product specific data.

Non-Target Organism Testing

The unique nature of biochemical pesticides has led to a reduction in the data requirements for these products, as compared to synthetic chemical pesticides. Maximum hazard or limit dose testing of the technical grade of the active ingredient (TGAI) is used in assessing hazard to non-target wildlife. The TGAI is the purest and highest concentration form of the biochemical pesticide active ingredient.

There are three tiers of biochemical pesticide data requirements with regards to non-target organism testing. If adverse effects are not observed in Tier I testing (short term studies on non-target birds, aquatic organisms, plants, and insects), no further testing will be required. Should adverse effects be observed in Tier

I studies, Tier II environmental fate studies will be triggered. If Tier II studies indicate that the biochemical active ingredient will persist in the environment, potentially resulting in longer exposure periods, longer term Tier III non-target wildlife studies will be required. Rarely are biochemical pesticides subjected to testing above Tier I.

Once the potential hazards to non-target wildlife have been determined via the tiered testing scheme, risk to non-target wildlife can be assessed based on expected exposure to a biochemical active ingredient via its application in an end-use product (EP) according to its proposed product label use directions.

Product Performance

Product performance data must be developed for all biochemical pesticides. However, such data is typically not required to be submitted unless it relates to a public health pest or is requested by the agency.

Microbial Data Requirements

Product Analysis and Mammalian Toxicology

Crucial to any evaluation of the hazards presented by a microbial pest control agent is correct identification. This identification allows the Agency to ascertain possible hazards associated with the proposed microbial agent and any closely related organisms, and to utilize published literature to facilitate the review. The Agency expects a registrant to provide the most accurate, current taxonomic information to verify the identity of their active microbial agent. For bacteria this information can include genetic DNA homology, morphology, biochemical tests and antibiotic sensitivity. Information for other types of microbes such as fungi, viruses and protozoa is usually less extensive, and may therefore involve other identification methodologies such as serotyping, DNA homology, restriction mapping or isozyme analysis when available. Any adverse effects known to be associated with the microbe, or closely related species, (such as toxin production and pathogenicity in species other than the target pest) should also be reported.

Additionally, the method used to manufacture microbial products is examined to determine whether adequate quality controls are in place to insure a pure product. This quality control review includes an examination for methods to verify purity and stability of the seed or stock cultures and to ensure that the final product is not contaminated with mammalian pathogens. Consideration is also given to final quality control measures for the microbial product that determine potency to insure that these tests relate to bioactivity and label claims (13).

The purpose of reviewing mammalian toxicology data for microbial pesticides is to ensure that the use of these products causes no unreasonable adverse effects to human health or non-target mammals. In order to do this the Agency must verify that the microbial product is correctly identified, presents little possibility of pathogenicity or toxicity to humans or other mammals, and is manufactured in a manner to prevent contamination with human pathogens.

To assure the safety of microbial pest control agents toward mammalian species, the Agency has adopted a tiered testing scheme similar to the tiered scheme used for biochemical pesticides. Tier I is designed to expose the test animal, mice or rats, to a single acute, maximum hazard or limit dose of the live microbial pesticide. Tests involving mammalian tissue cultures are required for viral pest control agents to insure there is no possibility of mammalian infection given optimal conditions for expression of viral pathogenesis.

The major endpoints for the toxicity/pathogenicity tests are to observe any adverse effects on the test animals and to establish that the microbial test substance is being cleared from the exposed animals. The animals are observed for any unusual clinical signs during the test, and for gross abnormalities at necropsy. Specific organs are isolated from sacrificed animals during the course of the test to determine the level of microbial test substance present. This is done to assure that a high dose was administered and track the normal mammalian response which recognizes the test substance as foreign and clears it from the system. Unusual persistence of the test microbe in an organ is also considered an adverse effect. Replication of the test microbe in organs is also an adverse reaction, indicating potential for infectivity.

If any adverse effects are noted in the Tier I of the toxicity/pathogenicity tests, further testing is indicated using a tier progression to verify the observed effects and clarify the source of the effects. These Tier II tests could involve a subchronic toxicity/pathogenicity test or, if the adverse effect was believed to be due to a toxic reaction rather than pathogenicity, an acute toxicity test to establish an LD50 value for the toxin. Residue data are required if significant human health concerns arise from the toxicology testing. The majority of biopesticide products screened to date have not indicated any adverse effects to warrant testing further than Tier I.

In addition to testing the safety of the purified microbial agent, the safety of the marketed pesticide product, including inert ingredients, is ascertained. Acute oral, dermal, and inhalation toxicity as well as eye irritation, and dermal irritation testing may be required. However, rationales for no further testing may be appropriate depending on the nature of the inert ingredients and results of the initial toxicity/pathogenicity tests with the microbial agent. Any incidents of hypersensitivity in production workers, applicators or the general public must be reported to the Agency.

Genetically Modified Microbial Pesticides

Genetically modified microbial pesticides may be subject to different data or information requirements on a case-by-case basis, depending on the particular microorganism, the parent microorganism, the proposed use pattern, and the manner and extent to which the organism has been genetically modified. Additional data requirements may include:

- Information on the genetic engineering techniques used;
- The identity of the inserted or deleted gene segment (base sequence data or enzyme restriction map of the gene);

- Information on the region controlling expression of the gene in question;
- A description of the new traits or characteristics that are intended to be expressed;
- Tests to evaluate genetic stability and exchange of the new traits; and/or
- Selected Tier II environmental expression and toxicology tests.

It is important for applicants to work closely with the Agency regarding data requirements to ensure that the proper tests are done and any unique characteristics of the microbial pesticide are taken into account in specific testing procedures.

Non-Target Organism Testing

The unique nature of microbial pesticides has led to changes in the data requirements for these products as compared to synthetic chemical pesticides. This is particularly evident in assessing risk to non-target wildlife (14). The testing requirements have been set up to test not only toxicity but also pathogenicity. This was accomplished by increasing the length of the tests (up to 30 days), and looking for signs of infection during and after the testing period. Beneficial insect testing was added in order to ensure that potential risks from insect pathogens used as pesticides had been adequately assessed.

For microbial pesticides used to control post-harvest diseases, the non-target organism data requirements to assess potential risks would also follow this case by case procedure. For example, in many instances the use of these products would be in enclosed areas (i.e., packing houses, storage buildings, etc.) and would be considered an indoor use. If this were the case, then testing of non-target organisms would probably not be required because of a lack of exposure. However, if the proposed use was determined to be outdoor and to have potential exposure to non-target organisms, then the ecological testing requirements would need to be addressed.

Tier I short term testing utilizes maximum hazard or limit dosing of non-target organisms. If no adverse results are observed in Tier I, then further testing is not warranted and environmental fate data are not required. In the first tier of non-target organism testing, avian oral, freshwater fish, freshwater aquatic invertebrate, and honeybee testing are required. In addition, tests to evaluate microbial pesticide effects on wild mammals, plants, and beneficial insects are required depending on the proposed use site, target organism, and degree of anticipated exposure. If adverse effects are observed in the first tier, then potential exposure to non-target organisms is evaluated in Tier II studies.

Product Performance

Product performance data must be developed for all microbial pesticides. However, such data is typically not required to be submitted unless it relates to a public health pest or is requested by the agency (15).

Plant-Incorporated Protectant Data Requirements

In general, the data requirements for PIPs are based on those for microbial pesticides (16). The reason for this situation is that PIP traits registered to date have been developed from genes found in microorganisms. The exact data requirements for each product have been developed on a case by case basis. The majority of products EPA has seen have been proteins, either related to plant viruses or based on proteins from the common soil bacteria *Bacillus thuringiensis* (*Bt*). The general data requirements include product characterization, mammalian toxicity, allergenicity potential, effects on non-target organisms, and environmental fate. For the *Bt* products, insect resistance management is included to prevent the loss of benefits of both the microbial sprays and the *Bt* PIPs from overuse and selection for resistant pest populations.

Conclusion

EPA is committed to encouraging the development and use of low risk biological pesticides as alternatives to conventional chemical pesticides. This commitment is shown by having a division dedicated to the registration of biopesticides, as well as distinct review timelines, fees, and required data. The efficient, effective approval of safer pesticides as well as a transparent, predictable process in decision making are top priorities for EPA, OPP, and BPPD.

Every day, the management and staff of BPPD focus on protecting human health and the environment by reducing the risks of pesticides through regulating biopesticides and encouraging pollution prevention practices. These safer options maximize the benefits of pesticides while helping to protect the air we breathe and the water we drink for generations to come. EPA looks forward to a continued role in helping to bring a broad array of safer pesticide options to market.

References

1. U.S. Environmental Protection Agency, Office of Pesticide Programs. *Biopesticides Fact Sheet*, EPA 731-F-08-009, October 2008.
2. Mendelsohn, M.; Kough, J.; Rose, R. Biological Pesticides: How Reduced Risk Applies. In *Eighth IUPAC International Congress of Pesticide Chemistry Book of Abstracts Volume 2*; July 4–9, 1994.
3. U.S. Environmental Protection Agency, Office of Pesticide Programs, Web Page FIFRA; <http://www.epa.gov/pesticides/bluebook/FIFRA.pdf> (accessed June 26, 2014).
4. U.S. Environmental Protection Agency, Office of Pesticide Programs, Web Page FFDCA; <http://www.epa.gov/pesticides/regulating/laws.htm#ffdca> (accessed June 26, 2014).
5. U.S. Environmental Protection Agency, Office of Pesticide Programs, Web Page FQPA; <http://www.epa.gov/pesticides/regulating/laws/fqpa/index.htm> (accessed June 26, 2014).

6. U.S. Environmental Protection Agency, Office of Pesticide Programs, Web Page PRIA; <http://www.epa.gov/pesticides/fees/tool/category-table.html> (accessed June 26, 2014).
7. U.S. Environmental Protection Agency, Office of Pesticide Programs, Web Page Section 18s; <http://www.epa.gov/opprd001/section18/> (accessed June 26, 2014).
8. U.S. Environmental Protection Agency, Office of Pesticide Programs, Web Page The Pesticide Registration Manual; <http://www.epa.gov/opprd001/registrationmanual/> (accessed June 26, 2014).
9. U.S. Environmental Protection Agency, Office of Pesticide Programs. *Pesticide Registration Manual Chapter 3: Additional Considerations for Biopesticide Products*; <http://www2.epa.gov/pesticide-registration/pesticide-registration-manual-chapter-3-additional-considerations> (accessed June 26, 2014).
10. U.S. Environmental Protection Agency, Office of Pesticide Programs, Web Page Joint Reviews; <http://www.epa.gov/oppfead1/international/naftatwg/biopest-jointre.html> (accessed June 26, 2014).
11. Mendelsohn, M.; Ellwanger, T.; Rose, R.; Kough, J.; Hutton, P. Registration of Biologicals I How Product Formulations Affect Data Requirements. In *Biorational Pest Control Agents/Formulation and Delivery*; Hall, F., Barry, J., Eds.; ACS Symposium Series 595; American Chemical Society: Washington, DC, 1995.
12. Mendelsohn, M.; Nelson, W.; Jamerson, H.; Beegle, C.; Sjoblad, R. Regulatory Requirements for Biopesticide Registrations. In *Proceedings IR-4 Minor Use Registration and IPM: Registration of Biologically Based Pesticides for Minor Crops*; April 21, 1994.
13. Mendelsohn, M.; Hutton, P. Obtaining EPA Approval to Test or Commercialize Microbial and/or Biochemical Pesticides. In *Proceedings U.S. Department of Agriculture Interagency Gypsy Moth Research Forum I 995* (United States Department of Agriculture Forest Service Northeastern Forest Experiment Station General Technical Report NE-213).
14. Mendelsohn, M.; Delfosse, E.; Grable, C.; Kough, J.; Bays, D.; Hutton, P. Commercialization, Facilitation and Implementation of Biological Control Agents: A Government Perspective. In *Biological Control of Postharvest Diseases - Theory and Practice*; Wilson, C.; Wisniewski, M., Ed.; CRC Press: Boca Raton, FL, 1994.
15. U.S. Environmental Protection Agency, Office of Pesticide Programs, Web Page Public Health Pests; http://www.epa.gov/PR_Notices/pr2002-1.pdf (accessed June 26, 2014).
16. Mendelsohn, M.; Kough, J.; Vaituzis, Z.; Matthews, K. Are Bt crops safe? The U.S. EPA's analysis of Bt crops finds they pose no significant risk to the environment or to human health. *Nat. Biotechnol.* **2003**, 21 (9), 1 003–1009.