Pest Management Regulatory Agency
Agence de réglementation de la lutte antiparasitaire

Regulatory Directive DIR2001-02

Guidelines for the Registration of Microbial Pest Control Agents and Products

This directive outlines the requirements for the registration of microbial pest control agents and products proposed for pest management in Canada at this time. The Canadian data requirements are essentially harmonized with the United States Environmental Protection Agency. Microbial pest control agents are naturally occurring or genetically modified microorganisms, including bacteria, algae, fungi, protozoa, viruses, mycoplasmae or rickettsiae, and related organisms.

Several regulatory proposals, including PRO98-01, *Guidelines for the Registration of Microbial Pest Control Agents and Products*, dated January 30, 1998 and PRO93-05, *Research Permit Guidelines for Microbial Pest Control Agents*, dated November 25, 1993, invited comments on proposed registration requirements for microbial pest control agents. Approximately 65 detailed comments on PRO93-05 were received from interested parties in the biotechnology, agri-food and forestry sectors, and eight comments were received on PRO98-01 and incorporated as appropriate.

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Foreword

This directive outlines the requirements for the registration of microbial pest control agents and products proposed for pest management in Canada at this time. Microbial pest control agents are naturally occurring or genetically modified microorganisms, including bacteria, algae, fungi, protozoa, viruses, mycoplasmae or rickettsiae, and related organisms.

This directive reflects progress in several important areas:

- The Canadian data requirements are essentially harmonized with the United States (U.S.) Environmental Protection Agency (EPA). Canada accepts all U.S. guidelines for conducting studies. Both countries require efficacy data to be generated. International activity involving the Organisation for Economic Co-operation and Development (OECD) Working Group on Pesticides has developed a document, *Guidance for Registration Requirements for Microbial Pesticides*, which will be published in 2001. The document proposes guidance for registration requirements for microbial products, and indicates where there are differences among countries.

- The Guidelines will help enable products that have the potential to contribute to alternative pest management to be considered for registration because the data requirements are specifically developed for these types of products.

- The Guidelines support effective and sustainable pest management and the introduction of new pest management technology, fundamental elements of the Pest Management Regulatory Agency's (PMRA) overall program to reduce risk to humans and the environment.

Several regulatory proposals, including PRO98-01, *Guidelines for the Registration of Microbial Pest Control Agents and Products*, dated January 30, 1998 and PRO93-05, *Research Permit Guidelines for Microbial Pest Control Agents*, dated November 25, 1993, invited comments on proposed registration requirements for microbial pest control agents. Approximately 65 detailed comments on PRO93-05 were received from interested parties in the biotechnology, agri-food and forestry sectors, and eight comments were received on PRO98-01 and incorporated as appropriate.

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1.0 Introduction

Microbial pest control agents (MPCAs) and end-use products (EPs) are subject to the *Pest Control Products Act* (PCPA) and Regulations, and the *Food and Drugs Act* (FDA) and Regulations. The PCPA, administered by the PMRA of Health Canada (HC), requires that all pest control products, including MPCAs and resulting EPs, be registered prior to being imported into, sold or used in Canada. Regulations made pursuant to the PCPA set forth general registration requirements and procedures.

The FDA regulates products, including the MPCAs and EPs, that may be adulterants of food. Regulations made pursuant to the FDA set forth allowable maximum residue limits (MRLs) for adulterative substances on food.

The purpose of these guidelines is to outline the scientific and technical information requirements for registration of a microbial product. These registration guidelines for MPCAs and EPs should be considered as a companion or guidance document to the legislation and to the *Registration Handbook for Pest Control Products Under the Pest Control Products Act and Regulations* (Registration Handbook). Applicants should carefully review this document and the Registration Handbook and consult with the PMRA prior to making a registration submission.

The registration requirements outlined in this regulatory proposal were developed to address the safety, merit and value of EPs. MPCAs include both naturally occurring as well as genetically modified bacteria, algae, fungi, protozoa, viruses, mycoplasmae or rickettsiae, and related organisms. The organization of the information parallels that for other pest control products but also addresses unique aspects of MPCAs, such as biological properties, host range, potential pathogenicity, infectivity and the abilities to persist, multiply and disseminate.

Because MPCAs represent a diverse range of microorganisms, not all studies or data requirements may be appropriate for a specific microorganism. Applicants should consider the unique characteristics of their microorganism when addressing specific data requirements and protocols, and are encouraged to consult with the PMRA before testing begins. In addition, waivers for certain data requirements will be considered when accompanied by a sound scientific rationale.

The PMRA and the EPA are committed to joint reviews and worksharing of microbial pesticide evaluations on a regular basis. Consequently, the PMRA and the EPA have established a process for the joint review of microbial pest control products for which the proposed use pattern is common to both countries. Joint reviews will 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 worksharing requires a shared understanding of the responsibilities of each agency, as well as common procedures and time frames. For additional information, please consult the document *NAFTA Technical Working Group on Pesticides: Procedures for Joint*

Review of Microbials and Semiochemicals, which can be found on the PMRA web site, accessed through Health Canada's web site.

Research permit guidelines for the MPCAs are undergoing revision. Researchers should consult the Regulatory Proposal PRO93-05, *Research Permit Guidelines for Microbial Pest Control Products*, during the planning phase of research to determine the regulatory requirement for research and the procedures to obtain regulatory approval.

2.0 Definitions

Active Ingredient or Microbial Pest Control Agent (MPCA): A microorganism (bacterium, alga, fungus, protozoan, virus, mycoplasma or rickettsia and related organisms) and any associated metabolites, to which the effects of pest control are attributed.

Data Code (DACO): Numeric code used to identify specific data requirements (scientific data and studies). Refer to Appendix I, *Data Code (DACO) Table for Microbial Pest Control Products*, for a complete list of DACOs for microbials.

Ecozone: Large and very generalized ecologically distinctive area based on the interplay of landform, water, soil, climate, flora, fauna and human factors. The boundaries between the ecozones should be viewed as transitional areas rather than distinct lines of demarcation (see Appendix VII, *Microbial Pesticide Ecozones of Canada*).

End-use Product (EP): An MPCA-containing product that has labelling that includes directions for direct use or application for its intended pest control purposes, and does not state that the product may be used to manufacture or formulate other pest control products. In some cases, an EP is identical to the manufacturing-use product (MP), the technical grade of active ingredient (TGAI) or formulation intermediate (FI) (see below). In other cases, an EP is formulated from the MP by addition of formulation ingredients such as UV stabilizers, spray adjuvants, suspending agents, carriers, encapsulating materials, wetting agents, or anticaking compounds, required to produce a product suitable for application in pest control. In many cases, a microbial EP is manufactured via an integrated formulation process, i.e., the MP used for formulation is not a separate registered product.

Formulation Intermediate (FI): A microbial preparation containing the TGAI to which other ingredients have been added, e.g., preservatives, stabilizers, diluents, to produce a microbial preparation suitable for use in the manufacture of an EP.

Genetically-Engineered Microorganism (GEM): A microorganism modified genetically through the use of in vitro recombinant nucleic acid technology, including the insertion of genetic markers.

Indigenous: An MPCA isolated from, or known to occur, in the ecozone(s) of intended use.

Maximum Residue Limit (MRL): The maximum concentration of a pesticide residue (in mg/kg) legally permitted in or on food or animal feeds.

Residues: The number of microbial organisms or identifiable parts thereof that are left on the target following application. Alternatively, where appropriate, residues may relate to a measurable quantity of a representative chemical component or metabolic by-product of the MPCA.

Technical Grade of the Active Ingredient (TGAI): A material containing the MPCA in question that is produced commercially, or on a pilot scale in a manner equivalent to the planned commercial process, and to which no ingredient intentionally has been added except for purposes of the MPCA growth or replication, or typical purification. The TGAI is considered to be the purest preparation resulting from a typical production process, and it is the preparation intended for distribution and/or formulation into an FI or EP.

Tiering: A multilevel or tier system of information requirements is a feature of the approach to environmental testing, i.e., Part 8, *Environmental Fate* (see Section 5, Part 8 of this document), and Part 9, *Environmental Toxicology* (see Section 5, Part 9 of this document). Initial submissions must include results for all required Tier I tests. Note that test requirements are determined after consultation with the PMRA. If this initial testing indicates no significant hazard, then further testing usually will not be necessary. If results of concern are observed in Tier I, however, then the appropriate information from Tier II may be required, with progression to higher tiers as necessary.

3.0 Pre-Submission Consultations Regarding Data Requirements

These guidelines contain information regarding the data requirements for a variety of MPCAs and EPs. Since the data required for registration depend on the identity and biological properties of the MPCA, the nature of the product and the intended use pattern, applicants are encouraged to contact the PMRA for pre-submission consultation(s) during the development phase of the product. The main objectives of these consultations are to determine the appropriate test substances, study protocols and data that may be required for the registration of a particular proposed product as well as the type of information required to support data waivers. Applicants should contact the Pest Management Information Service for information on appropriate contacts for pre-submission consultation.

Before consulting the PMRA, applicants should familiarize themselves with these guidelines and be prepared to propose information and data deemed appropriate to address the requirements outlined.

An information package (two copies) must be submitted at least 45 days prior to a proposed meeting date. In addition to a cover letter requesting a pre-submission meeting and a proposed agenda of the issues to be discussed, the information package should contain, at a minimum, the information requested in Section 5, Part 1, *Label, Product Profile, Proposed Use Pattern(s) and International Regulatory Status*, and Section 5, Part 2.7, *Characterization of the MPCA*, of these guidelines. Depending on the stage of development of the proposed product, short summaries of available information regarding efficacy, manufacturing processes, product specifications, safety to the environment and human health as well as scientific rationales to support proposed data waivers may be included. Proposed study protocols, if available, should also be submitted.

Appendix I of these guidelines contains a DACO Table that lists all data that may be required for the registration of a microbial product. Proponents should photocopy the DACO Table and note in the right hand column of the table, the information submitted in the pre-submission package. In consultations regarding proposed products in the latter stages of development, this column may also indicate which data are available or where waivers will be requested. Subsequent to the pre-submission consultation, the data requirements for the specific proposed product and use pattern will be recorded in a modified DACO table, and a copy supplied to the applicant. A copy of this table must be enclosed with the submission for registration.

4.0 Organizing and Formatting a Complete Microbial Submission Package

With the establishment of the PMRA and the consolidation of various agencies responsible for pesticide regulation, opportunities for improved efficiencies are being identified. One such opportunity is the comprehensive screening of submissions early in the registration process. Submission screening has contributed to a more efficient regulatory system by providing correctly formatted submissions for efficient reviewing through improved handling, tracking, storage and retrieval. Screening facilitates the identification of deficiencies to applicants early in the review process and ensures that only complete and acceptable submissions are allowed into the review stream.

(a) Submission Verification

Within seven days of receipt, all submissions are verified to ensure that fees, forms, labels and required information have been provided according to the Registration Handbook. Deficiencies will result in a submission being returned to the applicant at the applicant's expense. An applicant whose submission is verified is provided with an acknowledgement card that includes the submission number. **This number should appear on all correspondence dealing with that submission.** Once verified, submissions are forwarded for screening.

(b) Submission Screens

Upon receipt by the Submission Screening Section, a submission for a new MPCA or a major new use of a registered EP is screened within 45 days. If no deficiencies are identified, the submission is accepted and forwarded to the relevant science divisions for review. If deficiencies are identified by the Screening Officer, they are summarized in a letter to the applicant. The applicant has 45 days to address all the deficiencies. If there is no response, or if the response is incomplete or inadequate, the submission is withdrawn and returned to the applicant at the applicant's expense. The submission number is no longer valid. An applicant may resubmit the package as a new submission.

(c) Elements of a Complete Submission Package

Only complete submissions will be considered for review by the Agency. A submission is normally composed of a covering letter, application form, fees, a product specification form, various letters of support and authorization, draft label, index of supporting scientific data or studies and the scientific data. Data are either required (R) or conditionally required (CR), depending on the purpose of the submission. Please refer to Appendix II for a list of the required and conditionally required data of a submission. All data identified as required (as well as applicable conditionally required data) during the pre-submission consultation for a particular proposed product, must be addressed with appropriate information, references to previously submitted data, or requests for waivers. A copy of the DACO table outlining the data requirements must also be submitted (see Section 3, *Pre-Submission Consultations Regarding Data Requirements*, for more information).

4.1 Organization of Supporting Data

Supporting data for registration should be divided into ten general parts, as outlined below. The numbering of these parts is analogous to those of traditional chemical pesticides to facilitate data management within the Agency. Certain parts are not identified (Parts 3, 6 and 11), as they are not required for the EPs. Each major part is further divided into sub-parts, as described in this document and in the DACO table, Appendix I.

Part 0 Index
Part 1 Label, Product Profile, Proposed Use Patterns and International Regulatory Status
Part 2 Product Characterization and Analysis
Part 4 Human Health and Safety Testing
Part 5 Exposure Assessment

Part 7 Food and Feed Residues Requirements
Part 8 Environmental Fate
Part 9 Environmental Toxicology
Part 10 Value
Part 12 Comprehensive Data Summaries

(a) Requests for Waivers from Data Requirements

When required data are not submitted, a request for a waiver from the requirement to produce and submit these data must be supplied by the applicant. Such requests must be recorded in the index and supported by surrogate data or a scientific rationale in place of the data. The "comments" field of the index should be used to indicate the nature of the information, e.g., request for waiver, surrogate study, etc. The request for waiver with supporting rationale or surrogate data must be placed in the data binders under the appropriate DACO.

(b) Using the Same Data to Support More than One DACO

When data are used to address more than one DACO they need only be included under one DACO and referenced for the other DACO(s). When data are used to support a DACO requirement in more than one data part, however, they must be submitted in their entirety for both data parts, i.e., data cannot be referenced between different data parts, as a submission is divided among the reviewing divisions.

(c) Using Literature to Address Data Requirements

Legible photocopies of both the printout and relevant papers should be submitted to the PMRA. Each report should be submitted under the appropriate DACO, and indexed as per Appendix III, *Directions for Creating a Data Index*. The comments field of the index must be completed, referring to the study as 'published'.

When more than ten published references are included in an information package as the result of an extensive literature search, it is acceptable to summarize these papers in a report that includes the search parameters and a traditional index to the individual papers, with reference to the summary report as a single document in the index, under the appropriate DACO.

(d) Multiple or Additional Data

When two or more studies are submitted for a particular DACO, all data should be submitted under that DACO and be separated by divider pages with side tabs. Refer to Section 4.2, below, for instructions on labelling side tabs for divider pages. Standard operating procedures or other information (excluding foreign reviews) submitted for a particular DACO should be included under that DACO

and separated with divider pages. The "comments" field of the index should be used to indicate the nature of the information, e.g., published study.

When a study to be submitted does not correspond to a specific DACO, it should be submitted under the appropriate *Other Studies/Data* DACO within the pertinent data part, e.g., a standard operating procedure pertaining to Part 4, *Human Health and Safety Testing*, should be submitted under DACO 4.9, *Other Studies/Data*.

Foreign reviews should be coded under DACO12.5 as outlined in Appendix I, *Data Code (DACO) for Microbial Pest Control Products* and included at the end of the last binder for its related data part, e.g., a foreign review of toxicology data, Part 4, would be coded as DACO 12.5.4, but would be included at the end of the last binder for Part 4, *Human Health and Safety Testing* (see Section 5, Part 12.5 of this document).

4.2 Organization of a Submission Package

The components of a submission should be organized as outlined below. Separate packages should be submitted for each proposed product, i.e., each TGAI, MP, FI or EP. Each package should include a covering letter, application form, product specification form, fee form, fees, supporting documentation, label and index. Relevant data must be included with each submission, and except where the MP or TGAI is identical to the EP, a single package of relevant data may be submitted for related submissions. Submission components should be submitted either in an envelope or compiled in binders and boxed, as outlined below:

(a) Envelope

The following elements of the non-data component should be submitted in an envelope:

- **(*i*)** covering letter, forms as required, fee and supporting documentation (when a product specification form is required, five photocopies must also be submitted);
- **(*ii*)** two copies of the label (hard copy format);
- **(*iii*)** one copy of the index and label in electronic format (diskette) (refer to Appendices III and IV); and,
- **(*iv*)** one copy of the Material Safety Data Sheet (MSDS) for the proposed product and each formulant.

(b) Binders

Data parts and other information submitted under DACOs, i.e., waiver requests, foreign reviews, study screens, etc., should be organized in three-ring binders (8.5 × 11 inches, and varying widths as necessary to a maximum of 3 inches), as follows:

The following information should be included together in the Summary Binder in the order presented.

NOTE: the Summary Binder is not the same as the Comprehensive Data Summaries Binder. Comprehensive Data Summaries are to be submitted in a separate binder labelled as Comprehensive Data Summaries. Refer to the data requirements for Part 12, *Comprehensive Data Summaries* under Section 5, Part 12.7 of this document.

(*i*) copy of the covering letter
(*ii*) Part 0, Index (paper copy)
(*iii*) Part 1, Label (paper copy), product profile, and international regulatory status (i.e., Parts 1.1, 1.2, and 1.3)
(*iv*) summaries of Parts 4, 8, 9, and 10 (i.e., Parts 4.1, 8.1, 9.1, and 10.1): These summaries should also be included with their respective data parts under the appropriate DACO.
(*v*) MSDSs for the product and each formulant
(*vi*) DACO table: As a result of pre-submission consultation, the applicant should receive a copy of the data required for registration in tabular format that is a modified version of the DACO table (Appendix I). This amended DACO table must be included with the submission for registration.

Other data, i.e., Parts 2–10, should be organized in binders such that different data parts are not combined within the same binder. Where necessary, data parts may be organized in volumes, i.e., specific parts such as Toxicology may consist of a number of volumes. Each binder should be clearly labelled on both the cover and the spine as outlined below. Divider pages with side tabs indicating the DACO number should be used to separate data or DACOs. When more than one study is submitted for a particular DACO, the divider tab should also include a reference number that is used in the Table of Contents to indicate the location of a particular study in the binder, e.g., DACO 4.2.2-1 to indicate that this is the first study submitted under DACO 4.2.2. This referencing method should only be used in the data binders and not in the index.

Individual data or DACOs and attachments should be paginated logically, with consecutive page numbers beginning at page 1. All data and information submitted must be legible.

(c) Labelling of Binders

The following information should be on the cover and spine of each binder:

(*i*) Name of the registrant
(*ii*) Product name
(*iii*) Scientific name of the MPCA
(*iv*) Part number and title
(*v*) Volume number (of total number of volumes) for the particular part
(*vi*) DACOs included in volume
(*vii*) Date of submission

Examples of binder label information:

XYZ Biologicals Inc.
LEP BE-GONE flowable for forestry
Active Ingredient: *Bacillus thuringiensis* var. *kurstaki*, strain RL 99
Part 2, Product Characterization and Analysis
Volume 1 of 2
DACOs 2.1–2.7
May 19, 1996

XYZ Biologicals Inc.
LEP BE-GONE flowable for forestry
Active Ingredient: *Bacillus thuringiensis* var. *kurstaki*, strain RL 99
Part 2, Product Characterization and Analysis
Volume 2 of 2
DACOs 2.8–2.12
May 19, 1996

(d) Table of Contents

A brief Table of Contents for each binder should be included at the beginning of each binder. The Table of Contents should include the DACO (which also serves as the tab number for locating the study or information within the binder), DACO title, and study citation (author, year, title).

Example of a single entry:

4.3.5	Short Term Dermal	Hartley, M. and Murray, W. (1994) S-1234 (Technical Grade) twenty-one day dermal study in rabbit.

(e) Required Number of Copies of Data

Refer to Appendix V for guidance on the number of copies required for data parts and other information supporting submissions.

(f) Data Submission

A complete application package, including forms, fees, data, and supporting information and documentation, should be submitted directly to:

Submission Management and Information Division
Pest Management Regulatory Agency
Health Canada
A.L. 6605E1
2720 Riverside Drive
Ottawa ON K1A 0K9

When sending data in boxes, the weight of individual boxes should not exceed 15 kg (30 lbs). **Note: All required submission components must be submitted together. It is unacceptable to submit only some components initially, with an indication that more are to follow.**

5.0 Data Required for the Registration of Microbial Pest Control Agents and Products

Normally, separate data packages must be submitted in support of each product to be registered, i.e., the TGAI or the MP and each EP. The EPs manufactured using an integrated formulation process may be supported by a single data package, however, where the MP or the TGAI is identical to the EP.

Part 0 Index

The index must be submitted in both electronic and hard copy formats with the appropriate number of hard copies. All scientific data and studies submitted must be properly indexed. Foreign reviews, study protocols, comprehensive summaries, and requests for waivers accompanying or replacing data along with the scientific rationale or surrogate data must also be indexed and identified in the comments field. When previously submitted data are used in support of a new submission, the data must be fully referenced in the index, using the comments field to indicate when the data were submitted and the associated submission and registration numbers.

Separate indices must be submitted for each submission, i.e., the TGAI, the MP and each EP, unless the product is manufactured using an integrated formulation process. Please refer to Appendix III for directions on preparing electronic and hard copy indices.

Part 1 Label, Product Profile, Proposed Use Pattern(s) and International Regulatory Status

Part 1.1 Label

Instructions and requirements for the preparation of labels for pest control products are outlined in the Registration Handbook and in Appendix IV, *Directions for Creating a Draft Label*. It is recognized that not all of the detailed label information identified in the Pest Control Products Regulations is always applicable to microbial products. Applicants are advised to consult the Submission Management and Information Division, PMRA, for advice in this matter.

As a general rule, the human health and environmental precautionary statements should reflect results of hazard testing. Label recommendations regarding use rates, timing, application equipment and methods, as well as storage temperatures and shelf life must be supported by valid performance and storage stability data. Product guarantee should be expressed in appropriate units, as outlined in this document in Section 5, Part 2.9, *Disclosure of Ingredients*.

It is expected that microbial products may constitute an important component of integrated pest management (IPM) and integrated resistance management (IRM) strategies. Recommendations regarding use in the IPM and IRM may appear on labels, provided that valid data to support claims are submitted. See Section 5, Part 10, *Value Assessment*.

Part 1.2 Product Profile and Proposed Use Patterns

A profile of the MPCA or EP and a synopsis of the proposed use pattern(s) are required, including:

(*i*) taxonomic designation;
(*ii*) a brief description of the MPCA: pertinent biological and pesticidal characteristics, such as origin of the MPCA, host range, mode of action, potential pathogenicity or genotoxicity, etc.;
(*iii*) concentration range, i.e., minimum and maximum levels, of the MPCA in the TGAI and the EP(s), in appropriate units;
(*iv*) product type, e.g., bioinsecticide, biofungicide, etc.;
(*v*) product class, e.g., restricted, domestic, etc.;
(*vi*) formulation type, e.g., aqueous suspension, granular, etc., including reference to recommended diluents, spreaders and stickers, as applicable;
(*vii*) pest(s) or disease(s) controlled or prevented, etc.;
(*viii*) proposed site(s) of application, e.g., forests, greenhouses, residential areas, aquatic systems, etc.;
(*ix*) crops treated, as applicable;
(*x*) proposed rates of application, including dose and volume of formulation;

(*xi*) frequency and timing of applications: should be presented in relation to host or pest phenology, the IPM and IRM considerations, and for food and feed uses and time to harvest;

(*xii*) application methods and equipment, i.e., specialized or unique application methods or equipment should be described;

(*xiii*) potential for occupational and bystander exposure with reference to protective measures, as appropriate; and

(*xiv*) potential for exposure to sensitive non-targets of environmental or economic significance or to specific ecosystems, e.g., aquatic, marine, etc., with reference to risk mitigation measures, as appropriate.

Part 1.3 International Regulatory Status of the MPCA and the EP

The international regulatory status of the MPCA proposed for registration and related EPs should be reported, including:

(*i*) countries of registration;

(*ii*) dates registered or status of review;

(*iii*) indication if regulatory reviews from the EPA, the European Union (EU) or other OECD countries are available or submitted (these reviews should be coded as Part 12, *Comprehensive Data Summaries* and included at the end of the relevant data part; see Section 4.1, *Organization of Supporting Data*, for more information);

(*iv*) use pattern(s) registered; and

(*v*) trade names, if different from Canadian submission.

Part 2 Product Characterization and Analysis

(a) Introduction

The purpose of *Product Characterization and Analysis* is to provide guidance with respect to the information and data required to adequately characterize the MPCA and to substantiate the production of a consistent, safe, stable and biologically active product. Clear, accurate and detailed information should be provided to allow evaluators to verify the characteristics of the MPCA and the quality of the EP distributed in commercial channels. The extent of characterization, manufacturing and quality assurance information required may vary according to the nature of the MPCA, production methods, and product type. Early consultation to determine specific requirements is strongly recommended.

Applicants are reminded that the data requirements for Part 4, *Human Health and Safety Testing*, Part 5, *Exposure Assessment*, Part 7, *Food and Feed Residue Studies*, Part 8, *Environmental Fate* and Part 9, *Environmental Toxicology* are based on the prerequisite that the MPCA proposed for registration has been well characterized, and that sufficient information to support characterization has been provided in Part 2. Detailed reporting on

the MPCA characterization will significantly influence the nature and extent of data required for the assessment of potential hazards to human health and the environment, particularly in those cases where exemptions or waivers of data requirements are requested by the applicant.

It is expected that relevant information related to the MPCA characterization, quantification and product analysis will have been delineated prior to the initiation of safety studies.

In all cases, the information and data submitted must provide reasonable assurance that the microbial product distributed commercially is the same as that tested for safety and characterized in Part 2.

(b) Requirements

Part 2.1 Name and Address of Applicant

Part 2.2 Name and Address of Manufacturing Plant

Part 2.3 Name and Address of Formulating Plant (if different from Part 2.2)

Part 2.4 Trade Name

Part 2.5 Binomial Name (MPCA)

Part 2.6 Canadian Patent Status Information: number, date issued, and expiry date, if applicable.

Part 2.7 Characterization of the MPCA

The information requirements outlined in Section 5, Part 2.7.1, *Origin, Derivation and Identification of MPCAs*, and Section 5, Part 2.7.2, *Biological Properties of MPCAs*, are applicable to all MPCAs. Section 5, Part 2.7.3, *Characterization of MPCAs Derived Through Recombinant Nucleic Acid Technologies*, outlines additional characterization requirements for those MPCAs that are derived through recombinant nucleic acid technology.

All information supplied should be supported by relevant data or referenced scientific literature. Where reference is made to scientific literature, copies of the papers must be supplied. In cases where characterization information relies heavily on the scientific literature, the relationship of referenced strains to that proposed for registration should be well described. Bridging data to support relatedness is acceptable.

The date when characterization work was initiated and the time frame over which characterization studies were conducted should be reported. Information supplied on relevant phenotypic and genotypic properties should reflect those properties of the MPCA as it exists in the EP.

Part 2.7.1 Origin, Derivation and Identification of MPCA(s)

Accurate identification of the MPCA is a key component of safety assessment. The following information related to the history, derivation and identification of the microorganism should be supplied:

(*i*) taxonomic designation to the species or other appropriate level to show relatedness or to distinguish the MPCA from related microorganisms and to describe its relationship to known pathogens; the taxonomic designation should be based on current international standards and be supported by technical data, including test methods, rationale, criteria for identification and results of tests, to substantiate this designation. It is recognized that the level of sophistication of taxonomic methodology varies with the type of microorganism. The methods used to identify and classify the microorganism should reflect the best technology and methodology available for the particular group of microorganisms;

(*ii*) the source(s) of all other names, such as alternatives, synonyms and superseded names associated with the microorganism;

(*iii*) strain numbers, culture collection and company codes (where appropriate);

(*iv*) origin of the strain, such as environmental, clinical, food isolate and culture collection; description of isolation procedure, including exact geographical origin of the MPCA isolate; and history of the strain during its development;

(*v*) a description of how the strain was preserved and maintained during product development; and

(*vi*) for the MPCAs derived through classical mutation procedures or homologous gene transfer via natural mechanisms, such as conjugation or transduction, the following information should be provided:

- the taxonomic information for the parents used to derive the MPCA (described as in *i* above);
- complete details of the methods used to derive the MPCA;
- a description of the new traits or characteristics acquired and expressed;
- a phenotypic and genotypic comparison with wild-type parents and donors, as appropriate;
- information concerning the mutation rate or gene transfer frequency; and
- genetic stability, e.g., reversion tendency, rate of plasmid loss, of the altered chromosomal or extrachromosomal entity.

Part 2.7.2 Biological Properties of the MPCA(s)

The evaluation of an MPCA will be facilitated by providing, in as much detail as possible, information pertaining to the biological properties of the MPCA. For some microorganisms, much of this information may already be available in the published scientific literature. In these cases, a detailed literature review may be appropriate in lieu of newly generated data. The acceptability of published data will depend on a clear description of the relationship between the MPCA proposed for registration and the microorganism(s) referenced in the open literature. Regulatory officials should be consulted to determine if published information may need to be supplemented with results of testing of the MPCA in question.

Information on the following biological properties should be provided:

(*i*) natural occurrence of the microorganism, including information on its geographical distribution, preferred or obligate hosts, habitats, ecological niches and level of natural occurrence in the environment;

(*ii*) target organisms: pathogenicity, kind of antagonism to target hosts, infective or toxic dose, transmissibility, and information on mode of action, where available;

(*iii*) pest host range, including possible effects on species most closely related to the target pests;

(*iv*) description of the life cycle of the agent, including the various forms of the agent that may occur, including any significant differences in pesticidal, pathogenic or toxigenic characteristics of the various forms, where applicable;

(*v*) description of any plasmids or other extra chromosomal genetic elements involved in pesticidal activity, pathogenicity, toxicity, etc.;

(*vi*) relevant physiological properties, including growth parameters, e.g., temperature (minimum, maximum and optimum growth temperatures), redox potential (Eh), pH, nutritional dependence, susceptibility or tolerance to antimicrobial agents used in human or veterinary medicine and susceptibility to environmental factors, such as sunlight and desiccation;

(*vii*) a description of any unusual morphological, physiological, biochemical, pesticidal or resistance characteristics of the microorganism, if such characteristics are different from the classical description of the species or microorganism;

(*viii*) history of use of the MPCA and closely related strains or species;

(*ix*) relationship to known pathogens of plants, vertebrates, invertebrates or other organisms;

(*x*) a detailed discussion of the history and relationship of the MPCA to any known human dermatophyte, to provide a basis for a waiver of dermal infectivity testing that may be required under Part 4.2, *Infectivity and Toxicity*;

(*xi*) if the MPCA is closely related to a known, toxigenic human pathogen, demonstration that known mammalian toxins are not produced or are not present in final products. Details of methodologies and techniques used to ascertain presence or absence of toxins should be provided;

(*xii*) for fungi and actinomycetes, characterization data including: a discussion of the potential for genotoxin production, based on the relationship of the MPCA to genera and species known to produce genotoxins. If genotoxin(s) are known to be produced by related fungi or actinomycetes, a sensitive analytical technique(s), appropriate to the metabolite in question, must be used as a part of the characterization of the MPCA to determine the possibility of production of such a genotoxin(s) by the MPCA; and

(*xiii*) information regarding reported adverse effects of the MPCA related to human exposure. Research on adverse effects should be based on an extensive search of the published literature. The report should include a complete description of the databases examined, key words and any other relevant criteria used to conduct the search.

Part 2.7.3 Characterization of MPCAs Derived Through Recombinant Nucleic Acid Technologies

This section outlines additional characterization requirements for GEMs, those MPCAs derived through recombinant nucleic acid technology. Detailed characterization at the molecular level and relevant phenotypic comparison with unmodified recipient and donor are important in identifying potential risks associated with a particular modified organism.

It is recommended that the following points be considered in the design and development of recombinant microorganisms intended for use in commercial pesticidal products:

(*i*) The use of antibiotic resistance or other markers of clinical or veterinary importance should be avoided. Use of these markers may be acceptable, on a case-by-case basis.

(*ii*) Introduced or modified genetic material should not be situated on a readily mobilizable genetic element, particularly one with a broad host range.

(*iii*) Recipient microorganisms should be well characterized and should not be closely related to a microorganism known to be a pathogen.

(*iv*) Introduced or modified genetic material should be limited, where possible, to that required for the intended function.

(*v*) Techniques that permit selection of the recombinant MPCA in the presence of unmodified counterparts and related microorganisms should be developed, as appropriate to the MPCA in question.

The following areas related to characterization of the genetic manipulation are to be addressed. It is essential that the data and information submitted be of sufficient technical detail to permit unambiguous verification of the nature and expression of the genetic construct.

Part 2.7.3.1 Taxonomy and Characterization of Recipient and Donor Microorganisms

Taxonomic designation of recipient and donor microorganisms must be validated as outlined in Section 5, Part 2.7.1, *Origin, Derivation and Identification, of the MPCA(s)*. Characterization information, equivalent to that outlined in Section 5, Part 2.7.2, *Biological Properties of MPCA(s)*, should be provided for the unmodified recipient microorganism.

Characterization information on donor organisms should be provided where relevant to the nature of the genetic information transferred.

For microorganisms that are well characterized or commonly used, the taxonomic designation of the recipient and donor organism(s) may suffice. Consultation with regulatory officials is recommended to determine the extent of the information required.

Part 2.7.3.2 Construction of the Recombinant Microorganism

Precise details of all methods and manipulations involved in the construction of the recombinant microorganism must be provided. Where applicable, these should include:

(*i*) details of manipulations or modifications of introduced, intermediate and recipient genetic material;
(*ii*) description and characterization of vectors used;
(*iii*) method and site(s) of insertion, deletion, mutation or rearrangement of genetic material; and
(*iv*) details of manipulations of cellular material.

For ease of review, a detailed flow diagram, identifying donors, recipients, vector DNA, etc., and outlining the steps involved to obtain the final construct is recommended.

Part 2.7.3.3 Nature and Expression of Introduced or Modified Genetic Material

The data listed below may be required, depending on the nature of the introduced or modified genetic material and on whether the intended modification(s) could result in a significant increase in risk to human health and safety or the environment. The applicant should consult regulatory officials regarding the extent of characterization required for the modified MPCA in question.

All introduced genetic material must be well characterized as to source, identity and purpose. Depending on the nature of the genetic modification, restriction maps and sequence data of the introduced or modified genetic material and adjacent regions, in the final construct, may be required. If required, sequence data should be accompanied by analysis of the structure of the genetic information, including regulatory regions and features, e.g., consensus sequences, promoters, transcription terminators, potential open

reading frames, unusual structural properties, etc. In the case of modifications that involve deletions, rearrangements or site-specific, in vitro metagenesis, sequence data of the region before and after modification are required. Information on location, orientation and copy number of introduced or modified genetic material should be provided as appropriate.

It is recommended that a summary diagram, outlining key features of the final construct, accompany the text description above.

The phenotypic expression of the introduced or modified genetic material should be reported. Claims concerning expression, or lack thereof, must be well substantiated using the best technology available. The potential for expression of residual donor, vector or adjacent host genetic material, not directly related to the intended function, must also be addressed.

Where genetic manipulations are directed to altered regulation of native genes, the characteristics and level of gene expression should be compared with that of the unmodified parent.

Genetic manipulations that involve insertion of a target gene into a new genetic background or significant alterations of a native target gene may need to be accompanied by amino acid sequence data of at least the *N*-terminal region of the protein product, especially in cases where there is a need to substantiate clearly the modification and the genetic consequences. Protein products in both native and new genetic backgrounds should be well characterized with respect to conventional protein biochemistry. Data on the relative expression of the protein in native and new hosts should also be provided.

Information regarding genetic manipulations directed toward gene inactivation, attenuation or debilitation of strains should also be outlined.

Part 2.7.3.4 Phenotypic Characterization of the Modified Microorganism

The phenotypic profile of the modified microorganism, including comparison with the conventional or unmodified counterpart(s), as appropriate, should be reported. Particular emphasis should be placed on host specificity and relevant physiological characteristics.

Part 2.8 Manufacturing Methods and Quality Assurance

Manufacturing methods and quality assurance (QA) programs should be described in sufficient detail to validate claims regarding integrity of the MPCA as well as product specifications, including potency, ingredient limits, and acceptable contaminant levels, both prior to registration and during manufacture of products intended for commercial use.

(a) Preservation and Maintenance of the Production Strain

It is required that sufficient quantities of the registered MPCA production strain, i.e., master seed stock, be prepared and preserved as a means of ensuring purity and consistency of the registered MPCA over time. Multiple aliquots of the master seed stock should be prepared and stored to serve as working seeds for subsequent production batches. The master and working seeds are expected to be well characterized and to reflect the properties of the MPCA as reported in Section 5, Part 2.7, *Characterization of the MPCA*.

Details of the preservation method should be provided, including adequate demonstration that the preservation methodology employed does ensure integrity and consistency of the MPCA production strain. Criteria to define integrity and consistency, specific to the MPCA in question, should be reported. Quality control (QC) procedures, e.g., biological activity and phenotypic or genotypic properties, carried out on master and working seeds should be described, including criteria that determine their acceptance for use in production.

It is recognized that some MPCAs are not amenable to preservation by conventional methods. It may be necessary, for example, to maintain or passage production strains in living hosts or tissue. This is acceptable, provided that criteria to ensure consistency and integrity of the MPCA are developed and reported.

It is also recommended that a sample of the registered MPCA be deposited in an internationally recognized culture collection.

(b) Manufacturing Processes

Information regarding the manufacturing processes for technical active ingredients and the EPs is required. The individual steps in the process should be clearly outlined, with particular emphasis on critical process points and measures taken to ensure consistent quality and to limit extraneous contamination, both chemical and biological. These steps would include preparation of culture media and inocula, scale up of culture to production volume, pilot or commercial scale cultivation, harvest and concentration of active ingredient, processing of final culture, formulation methods, packaging and storage.

Description of production methods should also incorporate details of the manufacturing facilities, including the approach used for good sanitary state of the production unit, equipment and instrumentation employed, procedures for cleaning and sterilizing equipment, production vessels, transfer lines, etc., and time frames for each step.

(c) Quality Assurance

As an integral part of the production process, the QA program should be outlined. QA programs would normally incorporate elements, such as appropriate QC of raw materials and ingredients; effective process controls during manufacture to limit introduction of extraneous organisms and deleterious substances; and valid, reliable methodology to monitor the integrity of the active ingredient during production and to ensure consistent quality and safety of the final product.

The applicant should provide a table summarizing the critical QC tests and criteria, i.e., product release standards, that determine whether a batch of technical active ingredient, MP, or EP will be released for commercial use. Details of sampling programs, including procedures, sample size, frequency and statistical validity should also be included in the description of quality assurance. Measures to be taken when product release standards are not met should be reported.

QC testing may include, but is not limited to, integrity of the MPCA, product guarantee, contaminant screening, pathogen screening and other tests appropriate to the nature of the product. Representative QC data from five production batches must be submitted. Data on pilot-scale production batches may be acceptable.

QC data on commercial batches produced after registration must be maintained and submitted to the PMRA upon request. Any significant variation in product release specifications or contamination problems must be reported immediately.

To aid in review, it is requested that a detailed flow chart diagram(s), outlining production steps, critical process features and quality control measures accompany the text description of Manufacturing Methods and Quality Assurance.

Part 2.9 Disclosure of Ingredients

Part 2.9.1 Product Specifications

Every *Application for New or Amended Registration* of an EP must be accompanied by a complete and accurate *Product Specification Form*. The original of the form must be submitted with the Application for Registration, and a copy should be included in this section. Although the *Product Specification Form* is not required for technical active ingredient preparations, information equivalent to that outlined for EPs is required for all ingredients intentionally added to preparations of the microbial active ingredient during processing.

Information required includes type of formulation, nature and percent composition of each ingredient, including identity and purpose in formulation, and the name and address of the basic manufacturer. Since the production of the MPCAs is by nature a variable process, the variation in percent composition of the MPCA preparation and other formulation ingredients should be presented.

The applicant must provide MSDSs or manufacturer's specifications, as well as related technical information, on all intentionally added ingredients such as emulsifiers, diluents, stabilizers or preservatives.

If the toxicological characteristics of an ingredient in the formulation suggest a potential for human health or environmental hazard, a rationale should be submitted as to why its use in the formulation is not considered to pose a significant risk.

Part 2.9.2 Potency Estimation and Product Guarantee

The guaranteed amount of active ingredient or MPCA will normally be expressed in recognized units of potency or other expression of biological activity per unit weight or volume. The analytical methodologies used to determine and verify biological activity must be described in detail, including standardization, sensitivity, reproducibility and statistical validity. Representative data to validate the assay must be submitted.

For the MPCAs wherein potency units are not applicable, e.g., plant disease antagonists, active ingredient content may be expressed in terms of the MPCA units per unit weight or volume, as appropriate to the agent in question. A correlation of the MPCA units with biological activity, e.g., percent germination, percent inhibition, must be demonstrated. A label guarantee expressed in terms of viability or percent germination is acceptable provided that the guarantee is related in some valid fashion to product activity or efficacy. In principle, this could involve correlation of the MPCA units or content with laboratory bioassay data on target or surrogate hosts or field performance data.

In cases where recognized units of potency have not been defined, are not appropriate, or registrants are proposing new approaches to activity or guarantee measurement, the rationale for and validation of the assay must be submitted. The preparation and use of standards, reproducibility, and relevance to biological activity must be well described for new assays.

In some situations, more than one method may be required to adequately express product guarantee and the MPCA content. For example, the expression of guarantee of products containing *Bacillus thuringiensis* in terms of insecticidal protein content only, would not suffice; a correlation with biological activity is also required. Similarly, baculovirus product guarantees should correlate occlusion body counts with biological activity of the product by means of, for example, potency ratios determined by bioassay.

Part 2.9.3 Unintentional Ingredients

Impurities, contaminants or extraneous materials that are likely to occur in microbial products include: microbial contaminants, microbial toxins, allergens and other metabolic products; impurities in materials used in the manufacturing process; by-products from chemical reactions in the manufacturing process; fermentation residues; extraneous host residues from the production of intracellular parasites in cell cultures, whole animals or other living forms; and mutants, or alternate forms of the MPCA. The nature and incidence of contamination will depend on the type of MPCA, the production methods and the production environment.

A discussion regarding the formation or presence of unintentional ingredients that are likely to occur in the particular MPCA preparation should be provided. The impact of these unintentional ingredients on product quality, particularly integrity of the active ingredient, biological activity and possible effects on human health and environmental safety should be discussed, as appropriate to the nature of the MPCA and specifics of the production scenario. It is always the responsibility of the manufacturer to validate claims that the presence and level of unintentional ingredients will not be deleterious to product quality, safety, or effectiveness.

If there is a likelihood that impurities, contaminants or extraneous substances known to be a hazard to humans or other non-target organisms are likely to be present in EPs or during any intermediary stage of production, data must be provided to show that such substances do not occur, or occur at levels too low to represent a risk in the product intended for commercial distribution.

The approach, methods and rationale for detection and quantification of specified contaminants of concern must be described in detail. The information provided here may be used as a basis for determining the nature and extent of contaminant screening deemed routinely necessary for the EP.

Procedures designed to ensure purity or minimize levels of contaminants should be reported in Part 2.8, *Manufacturing Methods and Quality Assurance* (see Section 5, Part 2.8). It may be possible to establish acceptable limits for some contaminants in consultation with regulatory officials. Rationales and supplementary information relevant to establishing limits of contaminants should be reported here.

Part 2.10 Analytical Data and Methodology

Detailed methodologies and validated data related to detection, identification, enumeration or quantification of the active ingredient, related metabolites, impurities, contaminants or other ingredients may be required. The applicant should consult regulatory officials regarding the nature of specific tests that may be required for certain types of the MPCAs and products.

Part 2.10.1 Active Ingredient or MPCA

Appropriate methodologies for detection, isolation, enumeration, quantification of the entire microorganism, parts thereof, or specific chemical components or metabolites may be required, depending on the nature of the microorganism and product. It is possible that one or more methods, apart from those involved in potency estimation, may be necessary as a function of the purpose of the test. These might include: methodologies to distinguish the MPCA from other closely related strains, monitoring of the active ingredient or relevant metabolite during production, quantification of doses for infectivity and toxicity testing, and enumeration of viable forms of the MPCA in tissues.

For the MPCAs with well-characterized, genetic modifications, precise immunological or molecular methods to identify and distinguish the modified strain from closely related strain, revertants, etc., are required.

Part 2.10.2 Analysis for Microbial Contaminants

The levels of extraneous microorganisms in the EP should not exceed those consistent with product safety and performance. Based on information provided in Part 2.9.3, *Unintentional Ingredients*, it may be necessary to set limits for some microbial contaminants. It is recommended that suitable indicator organisms be routinely monitored in production samples to assess the hygienic state of the production facility and process. See Section 5, Part 2.9.3 of this document.

Potential microbial hazards should be assessed by the applicant, using methods and criteria consistent with international standards for food or related microbial products, e.g., supplements, and probiotics. International standards set by the International Commission on Microbiological Specifications for Foods (ICMFS) are recommended.

Claims concerning the nature and level of microbial contaminants must be supported by data from five production batches. Details and validation of methods used to assay for microbial contamination must be submitted. Since the nature of microbial formulations presents unique problems with respect to analysis for contaminating microorganisms, it is important that the validity, specificity, sensitivity and reliability of the detection method be reported.

Part 2.10.3 Analysis for Other Unintentional Ingredients

If a known toxic metabolite or hazardous substance may be present in a product, precise and detailed methodologies for identification and analysis of the substance will be required.

Part 2.11 Storage Stability Testing

The PCPA and Regulations require that storage stability of products be verified to ensure product performance and safety. These data will be used to determine an appropriate expiry date for most products. The following factors should be considered in the design of storage stability tests:

(*i*) maintenance of necessary physical properties of the formulation, as appropriate, e.g., suspendibility, wettability, viscosity, etc.;
(*ii*) maintenance of certified limits of biological activity (potency);
(*iii*) influence of relevant environmental parameters on product stability, such as moisture content, temperature, light, and pH, as appropriate to the nature of the product; and
(*iv*) preservative properties of the formulation, including presence of contaminants, where appropriate.

The EP and the technical product, if stored, should be tested over a suitable period of time and in accordance with typical storage and use conditions. The storage and shipping directions on the label must reflect and be supported by data generated during stability testing.

For EPs in which growth or metabolism in the product container is a prerequisite for biological activity, appropriate QC procedures must be in place to ensure limitation of hazardous contaminants. Data to support quality claims during storage may be required.

Part 2.12 Summary of Physical and Chemical Properties

The following physical and chemical properties, as applicable, for TGAIs and EPs are required:

(*i*) physical state;
(*ii*) density, bulk density or specific gravity;
(*iii*) viscosity;
(*iv*) corrosion character, i.e., oxidizing or reducing action; and
(*v*) suspendibility, wettability and moisture content.

Methodologies for measurement should be referenced as appropriate. Viscosity measurements should be carried out at temperatures that reflect storage and commercial or field working conditions.

Part 2.13 Characterization and Analysis Requirements for New EPs of Registered MPCAs

New EPs of the currently registered MPCAs require submission of Part 2, *Product Characterization and Analysis*, data in the following areas:

Part 2.7	*Characterization of the MPCA*, as necessary to validate equivalence of the MPCA to that first registered;
Part 2.8	*Manufacturing Methods and Quality Assurance*, relevant to the new EP and strain maintenance, as necessary to validate;
Part 2.9	*Disclosure of Ingredients*, product specifications, potency estimation and product guarantee, and unintentional ingredients, as relevant to the new EP;
Part 2.10	*Analytical Data and Methodology*, as relevant to the new EP;
Part 2.11	*Storage Stability Testing*, as relevant to the new EP; and,
Part 2.12	*Summary of Physical and Chemical Properties*, as relevant to the new EP.

Where procedures, manufacturing methods, quality control monitoring, etc., are similar to the original MPCA or EP submission, reference can be made to Part 2, *Product Characterization and Analysis*, of the original data package. Significant changes in manufacturing methods, quality control or analytical methodologies for technical and EPs must be reported as a supplement to the original, *Product Characterization and Analysis*.

There must be assurance that the current MPCA does not differ from the registered MPCA. Part 2, *Product Characterization and Analysis,* information, and the nature of the new formulation will be used to determine further requirements, if any, for registration of new EPs. Similarly, for new uses, additional safety data will not normally be required, unless the new use entails a significant increase in exposure. Applicants are encouraged to provide rationales regarding proposed regulatory requirements for new uses and new products.

Strain substitutions subsequent to initial registration cannot be made without prior notification and approval. In situations where applicants are proposing strain substitutions or the registration of new MPCAs closely related to their registered MPCA(s), claims regarding relatedness should be supported by scientific rationale, including criteria for equivalence developed by the applicant. Bridging data to demonstrate equivalence or relatedness are acceptable.

Since criteria for equivalence or relatedness will vary depending on the nature of the MPCA, early consultation with regulatory officials is recommended.

Part 3 Chemistry for Registration of an EP or MA
(not applicable to microbial EPs)

Part 4 Human Health and Safety Testing

(a) Introduction

It is recommended that the characterization data requirements outlined in Section 5, Part 2.7, *Characterization of the MPCA*, be completed before safety testing is initiated. All components of the formulated product must be identified by quantity, purpose and source (see Section 5, Part 2.9.1, *Product Specifications*). It is essential that adequate quality control procedures be in place to ensure that each lot of the active ingredient and formulated product is equivalent to the active ingredient and the formulation that were safety tested (see Section 5, Part 2.8, *Manufacturing Methods and Quality Assurance*).

(b) Purpose

The purpose of the safety testing is to determine:

(*i*) the pathogenicity potential of the MPCA;
(*ii*) the infectivity or pattern of clearance of the MPCA; and
(*iii*) potential toxicological effects of the MPCA and any associated by-products.

The components of safety testing required to determine potential adverse effects to humans assume that the MPCA proposed for registration has been characterized to the extent possible and details are provided in Section 5, Part 2.7, *Characterization of the MPCA*. The evaluation of the product for human safety will be based both on the characterization data and on the data generated from studies required under this Part. All available data on product safety should be submitted. Subsequent to the review of submitted data, however, additional data may be requested.

Various types of tests are used to assess treatment-related effects via possible routes of exposure. While it is recognized that infectivity, pathogenicity and toxicity involve a complex set of host and microorganism interactions, it is believed that the following set of tests, in conjunction with the MPCA and product characterization and analysis, will provide a basis for evaluation of the safety of the MPCA with regard to infectivity, pathogenicity and toxicity. It is strongly recommended that the PMRA be consulted prior to initiating testing to determine specific data requirements and experimental protocols.

(c) Approach and Rationale

It is recognized that the full complement of tests may not be justified for all products and that all endpoints may not be necessary in all data. Where appropriate scientific data or rationales are available, the registrant is encouraged to request a waiver for those data that they feel are not justifiable, and to submit this request with the appropriate supporting information.

If the data from characterization and toxicology testing demonstrate no effects of concern, no further testing is required. Where the data indicate otherwise, further investigation will be required as follows:

(*i*) non-food-use products: specific tests required will be determined on a case-by-case basis after a detailed review of the submitted data has been completed; and

(*ii*) products used in or around food: if the characterization and toxicology test results indicate the presence of a mammalian toxicity hazard through oral exposure, a zero tolerance will be the initial position. If the applicant chooses to pursue the registration, then the product would be viewed as being similar to a conventional chemical pest control product and appropriate data would be required to establish an MRL.

The rationale for the data chosen for testing, protocol recommendations, and minimum data requirements are summarized below. It is strongly recommended that the registrant consult with the PMRA before commencing testing to ensure that the protocols chosen are acceptable.

It is expected that new uses and new formulations of the registered MPCAs will utilize the existing database, developed in accordance with these guidelines whenever possible. There must be assurances that the MPCA does not differ substantially from that currently registered (see Section 5, Part 2.13, *Characterization and Analysis Requirements for New End-Use Products of Registered MPCAs*.)

(d) Substance to be Tested

It is necessary to determine the form and purity of the MPCA in the test substance. In general:

(*i*) The MPCA used in data given in this part should be identical or equivalent in form and growth stage, and phenotypic and genotypic trait(s) to the product that is to be registered and to be tested for all other parts of the registration requirements.

(*ii*) The test material should be from the same lot throughout the duration of the data. If this is not feasible, all lots of the material to be tested should be as nearly identical as practical.

(*iii*) If contamination is probable, each lot of test sample should be tested for composition; identification and limits of microbial and other contaminants should be established before testing.

(*iv*) The type of test substance to be used in individual tests is given in Appendix VI.

(e) Dose

The appropriate dose of the test material for the above data is established by considering such factors as, the characteristics of the organism being tested, the application method used, the potency and concentration of the product, and the maximum exposure concentration.

(f) Study Requirements

The pre-test viability or activity of the test material must be stated. Microbial contaminants must be identified and levels quantified. The level of these contaminants should be reported to the fullest possible extent. The observation period after dosing should be at least 21 days. This duration is flexible and dependent on the type of microorganism and the pattern of clearance from the test animal. For infectivity testing, it is emphasized that absolute clearance or elimination of the test organism need not be demonstrated. An indication of a steady decline in the number of organisms detected in the test animal is required. A sufficient number of animals must be treated to allow for adequate controls and for interim sacrifice for infectivity determination. Appropriate measures must be taken to minimize the potential for contamination or cross-contamination.

Investigations are to include the following data:

(*i*) a detailed clinical examination of all animals made at least once daily;

(*ii*) body weight measurements taken just before test material administration, weekly thereafter and at scheduled or unscheduled death;

(*iii*) a gross necropsy of all animals should be performed at scheduled or unscheduled death and all gross pathological changes should be recorded. Microscopic examination of target organs, or organs showing evidence of gross pathology, should also be considered since it may yield useful information;

(*iv*) for all infectivity studies, infectivity or persistence assessed by a suitably sensitive method to detect the presence of the MPCA in tissues, organs and body fluids. The percent recovery and sensitivity of the detection method should be reported;

(*v*) the pattern of clearance for all infectivity studies. The MPCA should be enumerated for the following tissues from animals sacrificed at suitable intervals: liver, spleen, kidneys, heart, brain, blood, gastrointestinal tract, lungs, mesenteric and mediastinal lymph nodes, intraperitoneal fluid, and where appropriate, from lesions and from the inoculation site. Other tissues, organs and body fluids may have to be examined as indicated by the nature of any toxic and pathogenic effects

observed. A sufficient number of animals for interim sacrifice must be included to establish a pattern of clearance adequately;

(*vi*) for acute oral infectivity and toxicity studies, feces from the test animals should also be collected after dosing and at regular intervals during the study period to establish the clearance pattern;

(*vii*) organ weights; and

(*viii*) data obtained from control animals dosed with "vehicle material" and, if available, historical control data.

All data should be consistent with the principles of Good Laboratory Practice (GLP). For additional information, please consult the PMRA Regulatory Directive DIR98-01, *Good Laboratory Practice*. The directive can be found on the PMRA web site, accessed through Health Canada's web site.

(g) Waiver Requests

It may be that not all data or all endpoints in a particular study are required, either because they may be inappropriate for the product being tested, or because they have been adequately addressed in one or more of the other study protocols. The registrant is encouraged to request a waiver for these data or endpoints and to submit appropriate rationale and scientific data.

(h) Data Requirements

The MPCAs or EPs proposed for registration are subject to the testing requirements as outlined in Appendix VI.

Part 4.1 Summary

Part 4.2 Infectivity and Toxicity

The purpose of these tests is to assess the capability of the MPCAs to cause disease and tissue injury by invasion of, and multiplication within, human tissues (infectivity), and to produce toxins (toxicity).

Infection of tissue(s) can result in overt clinical disease, in latent infection that could be activated at any time, or in a carrier state in which viable organisms are shed from a clinically normal individual.

Toxicity may be associated with toxin production, metabolic product or by-product(s) or cellular components of killed and lysed organisms.

The infectivity tests are carried out on the MPCA proposed for registration. Dermal infectivity testing will not routinely be required. If the characterization data indicate that the microorganism is closely related to a known dermatophyte, however, dermal infectivity testing may be required.

Part 4.2.1 Summary

Part 4.2.2 Acute Oral Infectivity and Toxicity

A single high dose of the TGAI is administered by gavage to each test animal.

Part 4.2.3 Acute Pulmonary Infectivity and Toxicity

Intratracheal instillation of a single high dose of the TGAI is the preferred method. Data conducted by conventional inhalation methodology may be acceptable.

Part 4.3 Acute Infectivity (IV or IP)

Part 4.3.1 Summary

Part 4.3.2 Intravenous Infectivity (Bacteria or Viruses)

A single high dose of the purest available form of the MPCA injected *intravenously*.

Part 4.3.3 Intraperitoneal Infectivity (Fungi or Protozoa)

A single high dose of the purest available form of the MPCA injected *intraperitoneally* is the preferred method. If the intravenous route is possible, based on the physical characteristics of the microorganism, then it should be considered.

Part 4.4 Acute Dermal Toxicity

A single high dose of the EP is applied to approximately 10% of the body surface area of each test animal for a 24-hour exposure period.

Part 4.5 Irritation

Microorganisms and their metabolic by-products, contaminants and formulation ingredients may all be potential dermal irritants that must be assessed in dermal irritancy testing.

The MPCAs can also reasonably be expected to act as mild, reversible ocular irritants. Generally, this hazard can be adequately addressed through appropriate precautionary labelling. This labelling should reflect the physical and chemical nature of the EP and should be developed in consultation with PMRA. Failure to comply with precautionary

ocular labelling recommendations will require appropriate conventional ocular irritancy data.

Part 4.5.1 Summary

Part 4.5.2 Dermal Irritation Study

The protocol for dermal irritation data in the *OECD Guidelines for Testing of Chemicals # A-404, Acute Dermal Irritation, 1993*, is considered adequate for assessing the irritation potential.

Part 4.6 Reporting of Hypersensitivity Incidence

Hypersensitivity data will not normally be required since most microorganisms contain substances that would elicit a positive response in test animals. All of the MPCA formulations will therefore be considered potential sensitizing agents, and an appropriate label statement will be required. Information about the occurrence of any type of hypersensitivity during production, testing and manufacturing is to be reported with the submission. Any incidents occurring after registration of the product must also be reported, giving details, including a description of the MPCA and formulation, frequency, duration and routes of exposure to the material, clinical observations, including the type of reaction noted, and other relevant information. This information will be used to develop appropriate precautionary statements on the label.

Part 4.7 Tissue Culture (Viral Agents Only)

(a) Rationale

Viral agents may have carcinogenic potential or may be infectious to mammalian cells. The purpose of the following tests is to assess the capability of the viral pest control agent for infection (overt, persistent, latent or abortive), transformation and toxicity. The applicant should submit information or data addressing all of these endpoints.

(b) Protocol

(***i***) **Substance to be tested:** The most infectious form (form that gives optimal infection in the susceptible cell culture or host organism) of the virus should be used. The virus should be titred by the most sensitive method available, and in the most susceptible host system (tissue culture or host organism).

(***ii***) **Cell lines:** For testing purposes, the following cell lines are recommended: one human cell line (such as WI38); one primary cell type (such as foreskin); and one primate continuous line (such as monkey CV-1). For a cell transformation assay, the primary Syrian hamster embryo (SHE/SAV7) system is recommended. The source and genetic stability of each cell line used should be provided.

(*iii*) **Toxicity:** Each cell line should be tested and the results reported for efficiency of plating (clonal survival) as a measure of toxicity.

(*iv*) **Transformation:** The oncogenic potential of the viral agent should be evaluated, utilizing the SHE/SAV7 assay system. Appropriate positive and negative controls must be used. Inoculated cell cultures should be observed for 21 days. This assay may not be required if it is conclusively demonstrated in the infectivity testing that the viral nucleic acid is not persistent in any of the cell lines tested.

(*v*) **Infectivity:** Each cell line should be exposed to high multiplicities of the most infectious form of the virus. The cell cultures should be observed for 21 days post-inoculation for cytopathic effects. The cells should be assayed for virus titre and for viral antigen and nucleic acid on days 1, 2, 5, 7, 14 and 21. The cell culture fluids should be assayed for infectious virus, using an appropriate susceptible host. Appropriate controls should be used (such as inactivated test virus as a negative control, and permissive cell line or host organism as a positive control).

The enzyme linked immunosorbant assay (ELISA), dot-immunobinding assays, protein blot immunoassay (Western transfer), or other similar sensitive assays are recommended for protein determination.

The dot blot hybridization, in situ hybridization, Southern hybridization and other sensitive assays are recommended for nucleic acid determination.

The sensitivity and limitations of each assay should be presented. Any cytopathic effects and viral replication observed in tissue culture should be described.

Part 4.8 Genotoxic Potential

Fungi are known to produce toxin(s) and metabolic by-product(s), some of which have genotoxic potential. If the characterization information/data indicate potential for the production of known genotoxin(s), an appropriate and sensitive analytical test, for example, high pressure liquid chromatography (HPLC), must be done to detect the presence of such genotoxin(s). This is an integral part of the characterization of a fungal product described in Section 5, Part 2.7.2, *Biological Properties of the MPCA(s)*.

Part 5 Exposure Assessment

The information on the use pattern and potential for exposure to workers and bystanders provided under Part 1.2, *Product Profile and Proposed Use Patterns*, will be used to recommend protective clothing and decontamination procedures. Further exposure information will generally only be required where the information provided is inadequate to satisfactorily address potential concerns.

It is recommended that individuals involved in manufacture and application should undergo a medical examination prior to exposure, and at regular intervals thereafter. Any significant clinical findings related to exposure are to be reported.

Part 6 Metabolism/Toxicokinetics Studies (not applicable to microbial EPs)

Part 7 Food and Feed Residue Studies

Residue data are used to estimate the exposure of humans and livestock to potential residues of the MPCAs that are proposed for use on food and feed crops, and to set and enforce the MRLs. Unless specifically exempted, use of the EPs on a food crop requires the establishment of a residue limit under the FDA and Regulations or, where appropriate, compliance with general regulation B.15.002(1) of the Food and Drugs Regulations.

(a) The MRL Exemption

When the characterization, i.e., Part 2, *Product Characterization and Analysis*, indicates the lack of potential for known mammalian toxin(s), and the acute oral toxicity test, i.e., Part 4.2.2, *Acute Oral Infectivity and Toxicity*, shows that there will not be a significant human health concern, then an MPCA will be recommended for exemption from the establishment of a residue limit under the FDA and Regulations.

If the presence of a mammalian toxin has been identified and the petitioner wishes to pursue registration for a specific purpose, however, the product will be subject to the same data requirements as a chemical pesticide, and appropriate data will be required to establish an MRL. Other potential health concerns associated with food and feed residues of the MPCA may require additional safety testing as determined on a case-by-case basis.

Part 8 Environmental Fate

(a) Purpose

Environmental fate testing is intended to demonstrate whether a MPCA is capable of surviving or replicating in the environment to which it is applied. Environmental fate tests further provide an indication of which non-target organism(s) may be exposed to the MPCA as well as provide an indication of the extent of exposure.

(b) Approach to Testing

The need for fate testing is dependent on whether adverse effects on non-target organisms are noted in Tier I or Tier II environmental toxicology tests. The extent of environmental fate testing, if required for registration, is based mainly on the nature of the MPCA, i.e., whether it is indigenous or non-indigenous to the ecozone(s) of intended use. Ecozones typical of geographical areas of use in Canada are illustrated in Appendix VII.

If results of Tier I environmental toxicology studies for non-indigenous and genetically engineered MPCAs, or Tier II for indigenous MPCAs, indicate that the MPCA has a narrow host range (i.e., the MPCA affects only the target pest) or that it produces adverse effects on a limited number of taxonomically related species (other than those of environmental or economic importance) then the probability of risk to other non-target organisms should be sufficiently low to not require environmental fate testing.

If results of Tier I environmental toxicology studies for non-indigenous and genetically engineered MPCAs, or Tier II for indigenous MPCAs, indicate that the MPCA has a broad host range, or that it produces significant adverse effects on a number of non-target species (including those of environmental or economic importance) then the probability of risk to non-target organisms would be of sufficient magnitude to warrant laboratory or field fate testing.

Environmental fate data may also be required for the MPCAs that are determined by the PMRA to present unique environmental concerns. Advance consultation with the PMRA is therefore recommended to determine whether environmental fate testing will be required.

If environmental fate testing is required, results of fate data should indicate the dynamics of the population fluctuation, i.e., growth or survival curves, of the MPCA. The experimental design should consider parameters, such as inoculum size, potential for blooms or regrowth, and physical parameters, such as relative humidity, pH and temperature. The results of environmental fate tests will be used to:

(*i*) judge whether concentrations of the MPCA at the time of application will produce a significant impact on non-target organisms; and

(*ii*) determine whether quantities of the MPCA will persist for an extended period of time above background concentrations, thereby allowing for continued or subsequent impact on non-target organisms.

(c) General Conditions

For all of the MPCAs requiring field testing, field data are to be undertaken only after the environmental toxicology data and laboratory environmental fate data, if required, have been reviewed by the PMRA, and the data indicate that the MPCA is unlikely to produce a significant impact on the environment in the ecozone where the study site(s) is located. (For reference see Section 5, Part 9, *Environmental Toxicology*, section *e)* Tier Testing, and Appendix VIII for timing). If field data are indicated by the results of the environmental toxicology and laboratory fate data, the applicant should consult with the PMRA prior to initiation of field testing to ensure that the endpoints proposed for study will provide sufficient results to assess the environmental fate of the MPCA under actual conditions of use.

(d) Test Substance

The information required in these guidelines must be provided for the specific MPCA to be registered. However, it is recognized that the information required to meet many of the testing requirements is that which is known about the MPCA from the published scientific literature or from results in unpublished reports. Such data may initially be submitted in lieu of newly generated data to support the registration submission. Applicants should ensure that for data submitted from the published or unpublished literature, enough detail is reported to allow the PMRA to reach an independent conclusion regarding the fate and persistence of the MPCA. In cases where there is little or no existing information available on the specific MPCA to be registered, surrogate information on closely related microorganisms, or the parental strains of genetically modified MPCAs, may be provided; the PMRA will review the surrogate information and determine its acceptability.

If there are no relevant data available, the applicant must conduct tests to obtain the necessary information required by the PMRA. If this is the case, then the MPCA tested should be identical in form and equivalent in stage of growth and phenotypic and genotypic trait(s) to the product:

(*i*) characterized in Part 2, *Product Characterization and Analysis*;
(*ii*) to be registered; and
(*iii*) tested in all other sections of the guidelines.

The substance to be tested will also depend on the type of study being conducted. For example, the MPCA, i.e., pure active ingredient, is to be used for pure culture testing, while the EP is to be used for small- and large-scale field testing. For other fate tests, different forms of the MPCA, such as the TGAI may be appropriate. The recommended form of the MPCA is specified in the testing requirements described below.

(e) Tier Progression

If results of environmental fate tests indicate that the MPCA is capable of surviving and persisting in the environment of intended use and that susceptible non-target organism(s) are likely to be exposed, then further testing may be required as shown in Appendix VIII, *Environmental Toxicology and Environmental Fate Testing Tiers*, and Appendix IX, *Environmental Toxicology and Fate Testing Requirements*.

(f) Fate Testing Requirements

Part 8.1 Summary

Part 8.2 Laboratory Studies

Laboratory studies are intended to determine:

(*i*) the optimum and range of physical factors, e.g., pH, temperature, light, oxidation-reduction potential, humidity, salinity, water activity, and osmotic pressure, and chemical factors, e.g., carbon, nitrogen and oxygen concentrations, and mineral and organic compounds, required for the growth and subsistence of the MPCA;

(*ii*) the persistence, multiplication, and dispersion of the MPCA under various environmental conditions and in various environments of intended use, e.g., terrestrial, freshwater, and estuarine or marine; and

(*iii*) if applicable, the ability of any modified genetic material in a GEM (genetically engineered MPCA) to transfer to other organisms or persist in the environment.

Information from these basic requirements is required to predict the potential exposure of non-target organisms to the MPCA under actual use conditions and to help define the environmental parameters, should additional field testing be required.

It is recognized that in some situations, it may be difficult or impractical to test the environmental fate of a MPCA in the laboratory if unusual conditions must be established to represent the environment of intended use or exposure. Consultation with the PMRA may be required to ensure that a suitable approach to testing is selected for the MPCA in question.

Laboratory fate studies on GEMs and the non-indigenous MPCAs must be carried out in research facilities that provide adequate containment and disposal procedures. Researchers are requested to consult the *Laboratory Biosafety Guidelines* (2nd Edition, 1996, Health Canada, Ottawa, Ontario, ISBN: 0-662-24214-9) for general guidance on laboratory safety procedures.

Part 8.2.1 Pure Culture Testing

Pure culture or in vitro testing is necessary for defining the various physical and chemical factors that are required for the growth and subsistence of the MPCA. Although data with pure cultures may not provide a wholly accurate representation of the growth and persistence of the MPCA in the environment, the data obtained will assist in delineating the limits for growth and subsistence. Most of this information is typically required for the commercial production of the MPCA and should be readily available to the applicant. However, if such data are not available, the applicant will need to generate the appropriate data.

Where survival, growth, or replication of the MPCA is known to be limited by certain physical or chemical factors, this information should also be provided.

Part 8.2.2 Microcosm Testing

Microcosm data are used to assess the biotic and abiotic interactions of the MPCA in the environment to which it is to be applied. In these data, the ability of the MPCA to grow and persist in an environment more representative of the area of release is determined. Additionally, these data can be used to provide an estimate of the ability of any genetic material introduced into a MPCA to transfer to other organisms or to persist *ex vivo* in the environment.

To address the possible effects that additives in a EP may have on the fate of the MPCA, microcosm testing should be conducted with the EP. If it can be demonstrated that the additives intended for the EP would not likely alter the fate of the MPCA in the environment, then microcosm tests may be conducted with the most convenient form of the MPCA available. However, if a form of the MPCA other than the EP is used in microcosm tests, a rationale for the test substance selected should be submitted.

Testing should consist of simulated field trials in the laboratory to assess the fate of the MPCA in various environmental media, such as soil, vegetation, leaf litter, water and sediment. The selection of both appropriate environmental media and compartment(s), i.e., terrestrial, freshwater, or marine or estuarine, to be tested, depends on the intended use pattern(s) and mobility of the MPCA. For example, a foliar application of an MPCA could involve studies with vegetation, soil, leaf litter, water, and sediments, whereas an application directly to bare soil could involve studies with soil only, or with soil, water and sediment.

Environmental media used for fate testing should be obtained from each pesticide ecozone of intended application (up to five). The sampled media should be identical to the environmental media used in environmental toxicology testing. It is further recommended that sampled media be collected and handled in a manner that minimizes disturbance, especially of the viable organic portion.

Various external factors in the environment also play an important role in determining the fate of a MPCA or any introduced, genetic material in the case of a GEM. Some of these factors will be predetermined by the environmental medium, e.g., soil type, soil pH, water hardness, salinity, etc., while others can be controlled or managed, e.g., temperature, soil moisture content, humidity, light intensity and quantity, oxygen concentration, water flow velocity, etc. All factors should be adjusted or manipulated to the greatest extent possible to approximate average in situ conditions found in the ecozone of intended use.

If possible, the MPCA should be uniformly distributed in the soil or water at a concentration equivalent to:

(*i*) 10^6 active units of the MPCA per gram of soil or water; or
(*ii*) 1000 times the expected environmental concentration of the MPCA, immediately following a direct application at the maximum label rate to a 15-cm layer of soil or water, whichever is greater or achievable. If uniform distribution of the MPCA is not possible, then the MPCA should be applied to the surface of the medium at a concentration equivalent to the above.

Part 8.3 Greenhouse Studies

As a bridge between laboratory testing and field studies, applicants may wish to conduct research trials under simulated outdoor conditions, i.e., in a greenhouse, to obtain a better understanding of the fate characteristics of the MPCA.

Owing to a wide variation in the design and operation of greenhouse facilities, under some circumstances, greenhouse studies will be considered equivalent to small-scale field trials and, as such, environmental data may be required in support of a research permit. The need for these data will be determined by the PMRA on a case-by-case basis, depending on the extent of environmental exposure anticipated from the greenhouse, and on whether the MPCA is non-indigenous to the ecozone in which the greenhouse is located. Environmental data will not be required for greenhouse studies using the indigenous MPCAs. Applicants are requested to refer to the data requirements for small-scale field trials indicated below in Section 5, Part 8.4, *Field Studies*, and in Regulatory Proposal PRO93-05, *Research Permit Guidelines for Microbial Pest Control Products* (or most current regulatory document available), for information on the types of information that may be required.

Part 8.4 Field Studies

As discussed above, field studies may be required for an MPCA that has a broad host range, i.e., affects more than just target organisms, or that produces significant adverse effects on environmentally or economically important non-target organisms.

Field studies on the environmental fate of the MPCA, including any modified genetic material in the case of a GEM, must be conducted using the EP. Field studies should address such aspects of environmental fate as:

(*i*) growth or subsidence (to environmentally acceptable levels);
(*ii*) persistence;
(*iii*) dispersion; and
(*iv*) transfer and persistence of modified genetic material, if applicable.

Small-scale field studies should be limited to small plots with a total area of less than 10 ha/ecozone. To assess the fate of the MPCA under actual conditions of use, field studies must be carried out in the ecozone(s) of intended use. Progression to larger scale field studies may be required but should not be undertaken until results of small-scale environmental fate and environmental toxicology studies have been reviewed by the PMRA.

Applicants are reminded that research permits are often required to perform field studies with MPCAs. Researchers should refer to Regulatory Proposal PRO93-05, *Research Permit Guidelines for Microbial Pest Control Products* (or most current regulatory document available), for information on data requirements for Research Permit applications. In general, environmental data are often required in support of research permit requests for genetically engineered and non-indigenous MPCAs, but for MPCAs indigenous to the ecozone of intended use, such data are required only when aerial application or large-scale releases are planned.

Part 9 Environmental Toxicology

(a) Purpose

This Part prescribes the data requirements for assessing the potential environmental hazards of a microorganism intended for pest control registration. Specifically, environmental toxicology testing is required by the PMRA to predict possible adverse effects of MPCAs and added ingredients, i.e., formulants, in EPs, on such broad groups of non-target organisms as birds, mammals, fish, arthropods, non-arthropod invertebrates, microorganisms, and plants. Possible adverse effects can be expressed in terms of infectivity, pathogenicity or toxicity, and hypersensitivity. Infectivity describes the capability of an MPCA to invade and persist in a viable state or multiply within or on an organism, with or without disease manifestation. Pathogenicity or toxicity is expressed as direct injury to an organism of an acute, subacute, or chronic nature, as a result of the actions of the MPCA or its toxins. Hypersensitivity refers to the potential for an MPCA to initiate severe local tissue damage via the immunological consequences of exposure.

(b) Approach to Testing

Canada has been divided into five distinct ecozones representing the major agricultural and forestry land areas where microbial pesticides are most likely to be applied (see Appendix VII, *Microbial Pesticide Ecozones of Canada*). Ecozones are based mainly on natural diversity and, therefore, they cut across political boundaries separating the provinces and territories and extend into the northern United States. The boundaries between ecozones are viewed by the PMRA as transitional areas, rather than distinct lines of demarcation. Ecozones are used by the PMRA to determine the extent and the nature of environmental testing required for both registration and experimental field trials. For example, a microorganism is considered indigenous if it has been isolated from, or is known to occur in the ecozone(s) of intended use. Conversely, a non-indigenous

microorganism is one that has not been isolated from, or is not known to occur in the ecozone(s) of intended use.

GEMs include microorganisms that have been modified through in vitro manipulation of genetic material. They are generally classified by the PMRA as non-indigenous microorganisms.

A four-level tiered approach to environmental testing of microbial pesticides is a feature of the environmental toxicology and fate testing requirements of these guidelines. This tier testing scheme is outlined in detail in Appendix VIII, *Environmental Toxicology and Environmental Fate Testing Tiers*, Appendix IX, *Environmental Toxicology and Fate Testing Requirements* and Appendix X, *Non-Target Toxicology Testing*.

Initial submissions from applicants for registration must address all testing requirements in Tier I. Tier I requires acute toxicity testing on up to seven broad taxonomic groups of non-target organisms. Tier II requires environmental fate (persistence and dispersal) as well as additional acute toxicity testing of MPCAs. Tier III requires chronic toxicity, i.e., life cycle testing, as well as definitive toxicity testing, e.g., LC_{50}, LD_{50}. Tier IV requires experimental field testing of toxicity and fate. If results of concern are observed in Tier I, then the appropriate information from Tier II may be required, with progression to higher tiers as necessary.

In general, if significant adverse effects on non-target organisms are identified in Tier I tests with an MPCA that is non-indigenous to the ecozone(s) of intended use, then Tier II environmental toxicology and environmental fate testing are required to determine effects of the MPCA on susceptible non-target species, exposed at lower concentrations than in Tier I, and fate of the MPCA under laboratory or actual use conditions. In the case of indigenous MPCAs that produce significant adverse effects on non-target organisms in Tier I, environmental toxicology testing, but no fate testing, is required at Tier II. Fate testing of indigenous MPCAs, under field or actual use conditions, is required only if significant adverse effects on non-target organisms are observed during Tier II toxicology testing. For any MPCA that produces significant adverse effects on non-target organisms in Tier II studies, Tier IV toxicology field testing is required to determine whether adverse effects are realized under actual use conditions. Tier III toxicology testing is reserved only for non-indigenous MPCAs that produce significant adverse effects on non-targets in Tier II.

(c) Rationale

When an MPCA is applied, large numbers are often spread over biotic and abiotic components of the intended target area or environment. The MPCA can also be expected to disseminate to off-target areas, or neighbouring environments, during and following application via drift, surface runoff and possibly through leaching. As a result, exposure of non-target organisms to the MPCA may be greater than under natural conditions in

terms of numbers of non-target organisms exposed, number of different species exposed, and concentration of the MPCA to which individual non-targets are exposed.

The intended area of application, coupled with the likelihood of exposure of selected non-target organisms, will primarily determine the extent of environmental toxicology testing required for MPCAs in Tier I. Although use pattern is also considered in selecting appropriate groups and species of non-target organisms to be tested, little distinction will be made between terrestrial and aquatic use patterns regarding likelihood of exposure, as certain terrestrial uses have the potential to adversely impact aquatic systems through drift, runoff or leaching.

Considerable judgement will, therefore, be exercised in employing the proposed use pattern as a criterion for determining the extent of environmental toxicology testing. For example, while many use patterns obviously entail direct application to aquatic ecosystems, e.g., mosquito, nuisance fish, and aquatic weed control, less obvious or borderline terrestrial uses may also be considered aquatic uses. Some examples that fall into the latter category are applications to forests, drainage ditches, riverbanks and partially aquatic crops. Widespread applications to major crops could also warrant expanded testing if these crops are grown near bodies of water. In borderline situations, the distinction between terrestrial and aquatic use patterns will be determined on a case-by-case basis.

(d) General Information

(*i*) Genetically Engineered Microorganisms: Environmental toxicology data requirements for EPs containing GEMs are similar to those required for MPCAs containing non-indigenous microorganisms as defined in Section 5, Part 8, *Environmental Fate*.

(*ii*) Selection of Non-Target Test Organisms: The non-target organisms selected for testing will depend mainly on the known characteristics, the host specificity, i.e., host range, and the proposed use pattern(s) of the MPCA. As a result, the PMRA cannot prescribe precise numbers and species of non-target organisms to be tested for every MPCA. Pre-submission consultation with the PMRA is highly recommended before commencement of toxicity testing on non-target organisms. Such consultations will aid applicants in the identification and selection of non-targets requiring testing. The following selection criteria should be considered, prior to consultation, to help the applicant identify groups of non-target organisms that may be needed to assess the toxicological hazard(s) of the MPCA.

Consideration should first be given to the characteristics of the MPCA and any known adverse effects associated with the MPCA. For example, if the MPCA is known or suspected to be a toxin producer or a pathogen, selection of test species should consider the most likely host species. If there is no evidence that an MPCA is a pathogen, but it has closely related species, perhaps in the same genus, that are

known to be pathogens, then selection of test species should again consider the most likely host species. When selecting the most likely host species, a centrifugal taxonomic approach should be considered to identify the Canadian test species most closely related to a known host. In this approach, the first group of non-target organisms selected are those with close phenetic, i.e., phenotypic or genotypic, or phylogenetic, i.e., evolutionary, relatedness to the target pest or host organism, followed then by testing on more distantly related organisms. For example, the initial non-target organisms selected for testing would be species from the same genus as the target organism, then testing would be expanded to include non-target species from the same family, order, etc., as the target organism. Host range studies undertaken by the applicant to fulfil efficacy data requirements in Part 10, *Value Assessment* (see Section 5, Part 10), may, in some cases, be suitable to support the initial non-target organism testing requirements. For genetically modified microorganisms, i.e., MPCAs modified through classical genetic techniques such as chemical metagenesis, conjugation or transduction or through in vitro or genetic engineering techniques, selection of test species should be based upon the characteristics of both the parental microorganism(s) and the modified genetic material.

The non-target organisms selected for testing should also include taxonomically related species that meet one or more of the following general criteria:

- are known, or are suspected of being able to be infected by the MPCA;
- are susceptible to pathogens closely related taxonomically to the MPCA; or
- are morphologically, physiologically or biochemically similar to the target, i.e., possess traits that are known to be significant in host choice and acceptance.

If there is no reason to suspect that the MPCA or its close relatives are pathogens or capable of producing toxins, then test species should be selected based upon the intended use area and use pattern of the MPCA. Special consideration should be given to non-target species closely related to the target host that are known or believed to occur in the ecozone(s) of intended use (see Appendix VII, *Microbial Pesticide Ecozones of Canada*). If there are any non-target species that are obviously expected to be exposed to high concentrations of the MPCA, e.g., utilize the affected target host as a food source, then these species should also be considered for testing.

Finally, representative species from some or all of the seven broad groups of non-target organisms specified in the section *Non-Target Organism Testing*, may require testing with the MPCA. Most of the recommended test organisms in these broad groups are generally found across Canada; they have some environmental or economic importance; and they have been used as test species for assessing the effects of a variety of environmental stressors. The submission of surrogate or existing data as well as waiver requests based on sound scientific principles will

be considered in lieu of data generated with the MPCA. Applicants should refer to (*xii*) *Surrogate and Existing Data* and (*xiii*) *Waivers*, below, for details.

(*iii*) **Test Methods:** The design of appropriate test methods will depend upon the characteristics of the MPCA and its intended use pattern. A sufficient number of test organisms must be treated to allow for adequate controls, for statistical analysis and interpretation of the data, and for interim sacrifice for infectivity determination, if applicable. The number of organisms in each test group will depend on the species to be tested, the expected duration of the study, the tier testing level and whether single or multiple groups are to be treated. For example, maximum hazard or single group testing of aquatic arthropods, e.g., *Daphnia magna*, in Tier I would typically require 50 arthropods while definitive or multiple group testing in Tier III would require about 20 arthropods per group.

For MPCAs that are pathogens, existing pathogenicity test methods can be used as guidance for providing test data. The specific test method used should match the infectivity requirements of the pathogen and host and should be capable of detecting both infection and disease symptoms. Where the MPCA is not a pathogen, applicants can rely on standard toxicity test methods designed for the assessment of chemical pest control products as guidance for providing data. Consistent with the maximum hazard approach to testing, the conditions of the test method should optimize the chances of detecting an adverse effect.

The laboratory practices used to develop test data should be consistent with the PMRA Regulatory Directive DIR98-01, *Good Laboratory Practice*.

(*iv*) **Test Substance:** MPCAs can be applied in any one of a combination of naturally existing forms. It is necessary to determine the form and purity of the MPCA that is to be tested to support the registration of each TGAI and formulated product EP. In general:

- the MPCA to be tested for effects on non-target organisms should be identical in form, equivalent in stage of growth, and equivalent in phenotype and genotype to the product to be registered and characterized in Part 2, *Product Characterization and Analysis*, and tested in all other parts of these guidelines; and
- the test substance should be from the same lot throughout the duration of the studies. If this is not feasible, all lots of the material to be tested should be as nearly identical as practical.

The form of the MPCA to be tested will also depend in part on the tier testing level. In general, the EP is the preferred test substance in all toxicology testing tiers. However, due to the high dosages or challenge concentrations recommended in Tier I and Tier II, and to a lesser extent in Tier III, the PMRA recognizes that the use of the EP may not always be feasible. Therefore, the TGAI of the MPCA can be employed in Tier I, II and III tests. In addition, any substances,

i.e., adjuvants or formulants, intended to enhance the virulence or toxicity of the MPCA should be tested along with the TGAI. In Tier IV actual or simulated field trials, only the EP should be used. If the TGAI of the MPCA and EP are identical, however, Tier IV testing may also be carried out with the TGAI of the MPCA.

(*v*) **Age of Test Organisms:** To optimize the chances for detecting an adverse effect, the most sensitive stage in the life-cycle of the test species should be tested. For birds, mammals and fish, sufficient immunological and physiological differences exist between immature animals and mature animals to suggest that immature animals are potentially more susceptible to infection and possibly to the effects of any toxin produced by an MPCA. Therefore, in keeping with the maximum hazard approach to testing in Tier I, the use of immature birds, mammals and fish is recommended.

For arthropods, non-arthropod invertebrates and plants, the life-stage most likely to be exposed, or to be susceptible, to the MPCA is a more appropriate age to test than a standardized life-stage. Therefore, test species from these non-target organism groups should be treated either at the time of most likely exposure in the field or at the time of most likely susceptibility.

(*vi*) **Methods for Detecting the MPCA:** Various methods may be employed to detect the MPCA. The detection method should be appropriate for both the organism, e.g., bacterium, fungus, etc., and the mode of action of the MPCA. For example, mortality may be appropriate for toxicity tests whereas infectivity tests often require sophisticated assessment methods for detecting sublethal pathogenic effects. Some detection methods may employ serological or nucleic acid technology.

(*vii*) **Maximum Challenge Concentrations:** Following an application, MPCAs and any associated toxins have the potential to increase in concentration in the environment via multiplication and in host and susceptible non-target organisms via infection or predation. Test organisms in Tier I should be exposed to a maximum hazard or Maximum Challenge Concentration (MCC) of the MPCA. For toxicity testing of most non-target organisms, the MCC will generally be based on some safety factor multiplied by the amount of the MPCA or its toxin expected to be available following application at the maximum recommended label rate. For avian toxicity testing, however, the MCC will be a function of some safety factor that will be based in part on the route of administration and the MPCA concentration in the TGAI.

In most cases, testing at one concentration, i.e., the MCC, is expected to be sufficient to evaluate adverse effects. Consequently, if no adverse effects are observed at the MCC, testing at lower concentrations will usually not be necessary.

In all cases, a rationale for the test concentrations chosen should be provided.

(*viii*) **Routes of Exposure:** Exposure routes are specified in each test guideline. If infectivity is the mode of pesticidal action, non-target test organisms should be treated using the route of exposure most likely to lead to infection. Otherwise, test organisms should be treated using the same route(s) of exposure that is anticipated to be the most significant route in the environment. For example, to select the most appropriate exposure route for testing birds consideration should be given to the potential for ingestion and inhalation.

(*ix*) **Duration of Test:** The duration of the observation period following exposure of a non-target organism to the MPCA is dependent on various factors, including mode of pesticidal action, and, therefore, is difficult to generalize in a guideline. The guidelines provide that the general duration for most Tier I tests be approximately 30 days. For most MPCAs, this should permit time for incubation, infection, and manifestation of adverse effects in the test organisms.

In the case of infectivity testing, the observation period should take into account the possibility of a longer time for infection to occur and manifest in the test organism. Some test species, notably arthropods, may be difficult to culture and the test duration may need to be adjusted accordingly. In any event, to demonstrate that the MPCA exerts no adverse effect(s), an observation period should be chosen by the applicant such that the data will clearly establish that the test organism has not been adversely affected by the MPCA, and that for infectivity tests, an indication of a steady decline in the number of MPCAs in the test organism, i.e., an obvious pattern of clearance, has been established. If it can be clearly demonstrated that the MPCA has no effects, including the possibility of an infection without the manifestation of visible effects, then the prescribed observation period can be reduced.

(*x*) **Control Groups:** For all tests, the activity level of the MPCA should be related to its pesticide capability by running parallel studies in which target pests or hosts are exposed to the MPCA, i.e., positive controls. Alternatively, the activity of the MPCA, in terms of viability, can be assessed by another technique, e.g., culturing on a synthetic medium. In either case, the activity of the MPCA used in each test must be equal to, or greater than the activity of the MPCA in the EP to be registered. In the absence of positive controls, the authenticity of negative results with non-target test organisms will be questioned.

For all tests, a negative, no-dosed control group of the non-target organism should also be run concurrently with the test group and the positive control. Additional controls may be required depending on the non-target group being tested. For tests on birds and mammals, a concurrent control group is required consisting of the active ingredient that has been inactivated in such a way as to preserve cellular integrity, and for birds dosed by the pulmonary route of administration, a contact

control in which two no-dosed birds are placed in the same pen as the dosed birds should also be run to determine communicability of the MPCA. For tests on non-target plants with herbicidal MPCAs, an additional no-dosed control group of the target, i.e., pest, plant is also required.

In all cases, organisms must be randomly assigned to control and treatment groups to prevent bias.

(*xi*) **Reporting of Data:** It is incumbent upon the applicant to submit all relevant studies required by the PMRA to assess the potential hazards of the MPCA to non-target organisms. Furthermore, for each submitted study, a complete and accurate description of the test procedures employed, and all relevant data and analysis of results necessary for the PMRA to reach an independent conclusion must be provided.

(*xii*) **Surrogate and Existing Data:** Original data performed by, or under the direction of the applicant using the MPCA of a similar or taxonomically related microorganism may be submitted in support of registration. MPCAs are to be considered similar only if they are taxonomically equivalent and their host ranges are comparable. Some variation in host range is acceptable so long as the variation can be attributed to differences in virulence rather than to host specificity. If test data on a different MPCA are submitted in support of registration, the applicant must demonstrate that the MPCA in the formulation to be registered would, with respect to effects, behave identically to the MPCA used in the submitted study.

For some non-target organisms, effects data with microorganisms closely related to the MPCA to be registered may already exist in the published scientific literature. Existing effects data with related microorganisms can be submitted to support the registration of the MPCA. Thus, actual testing with the MPCA may not be required if published studies adequately address the testing requirement. Published studies must contain sufficient information, however, to permit a detailed analysis of the methodology and results of the research performed. Applicants should be aware that if published studies are submitted in lieu of actual test data, additional or supplemental information on the effects of any additives, e.g., formulants, adjuvants, intended for the EP to enhance virulence or toxicity of the MPCA may be requested by the PMRA. Applicants should be aware that, regardless of the information provided in support of registration, the absence of data for a specific testing requirement will not be considered evidence that the MPCA has no adverse effects on that group of non-target organisms.

(*xiii*) **Waivers:** The data requirements specified in Section 5, Part 9, *Environmental Toxicology*, apply to a wide variety of MPCAs with diverse biological properties, e.g., viruses, bacteria, fungi, protozoa, algae, etc., and as such may not always be appropriate for every MPCA. Some MPCAs may have unusual characteristics or atypical use patterns that would make particular data requirements inappropriate

either because it would not be possible to generate the required data or because the data would not be useful in the evaluation of the MPCA's hazards. In cases where a data requirement appears inappropriate for the MPCA or EP to be registered, an applicant can request a waiver for that requirement. To request a waiver, the applicant must submit a written request to the PMRA. The request must specifically identify the data requirement for which a waiver is requested, explain why the data requirement(s) should be waived, describe any unsuccessful attempts to generate the required data, furnish any other information that may support the request and, when appropriate, suggest alternative means of obtaining data to address the concern that underlies the data requirement. The PMRA will waive data requirements on a case-by-case basis in response to specific written requests from applicants.

(*xiv*) **Biosafety Guidelines:** Laboratory or greenhouse studies on MPCAs that are either non-indigenous to the ecozone in which the laboratory or greenhouse is located or are GEMs, must be conducted in research facilities that have adequate containment and disposal procedures. Researchers are requested to consult the *Laboratory Biosafety Guidelines* (1996, 2nd Edition, Health Canada, Ottawa, Ontario, ISBN: 0-662-24214-9) for general guidance on laboratory safety procedures and requirements.

(e) Tier Testing

Effects testing on non-target organisms is to follow a tiered approach outlined in Appendix IX, *Environmental Toxicology and Fate Testing Requirements*, and summarized in Appendix X, *Non-Target Toxicology Testing*. The general strategy for testing each of the major non-target organism groups is specified in the section *Non-Target Organism Testing*. Consultation with the PMRA after each tier of testing is recommended as results from lower tier tests will determine the need for testing at higher tier levels.

(*i*) **Tier I:** For Tier I toxicology tests, non-target organisms should be exposed to an MCC of the MPCA so as to maximize chances for either infection or toxic and pathogenic effects. The MCC of the test substance, and route of exposure, are expected to vary for the different broad groups of non-target organisms requiring testing.

Regular observations are required to record mortalities and note any behavioural, pathogenic or toxic adverse effects. Gross necropsy, histopathological examination and culture and isolation should be performed on exposure site tissues and other organs or tissues showing anatomical or physiological abnormalities in adversely affected test organisms. In cases where tissue preferences are known or suspected, the tissues should be examined whether or not gross anatomical or physiological changes are seen. At study conclusion, a representative sample of the surviving non-target test organisms should be

examined to determine whether or not they were affected by the MPCA without the manifestation of visible adverse effects.

If significant adverse effects are observed in Tier I tests, then Tier II environmental toxicology testing is required. If the MPCA is non-indigenous to the ecozone(s) of intended use, environmental fate (see Section 5, Part 8, *Environmental Fate*) testing is also required. Testing at higher Tiers is generally not required if significant adverse effects are not observed in Tier I tests. However, additional testing may be required if it is determined that there is a potential hazard to non-target test species despite negative Tier I results. As an MPCA that produces significant adverse effects on some non-target species in Tier II tests could pose an unacceptable risk, submission of Tier III and Tier IV tests are not routinely required in an initial registration submission, but may be requested following the initial review of Tier I and Tier II test results.

(*ii*) **Tier II:** Generally, Tier II toxicology tests will be required for MPCAs that either demonstrate a wide host range or produce significant adverse effects on non-target organisms of environmental or economic importance in Tier I. In some cases, an adverse effect on a single important species in Tier I, e.g., honey bees, may be sufficient to trigger Tier II testing; whereas, in other cases adverse effects on a number of non-target species, presumably of lesser importance, may be needed to trigger Tier II testing. Tier II environmental toxicology testing will, therefore, be required on a case-by-case basis. Again, advance consultation with the PMRA is recommended before applicants proceed from Tier I to Tier II.

Tier II toxicology testing should be carried out on any non-target species adversely affected by the MCC of the MPCA in Tier I tests. The MPCA concentration used to challenge test organisms in Tier II is specified in each testing requirement and, in most cases, is lower than the MCC used in Tier I testing. In some cases, however, the Tier II challenge concentration may be identical to the one used in Tier I. For example, if the route of exposure is via the diet using maximally infected targets or the MCC is administered by intravenous or intraperitoneal injection. For routes of exposure where the Tier II challenge concentration is expected to be the same as that used in Tier I, Tier II toxicology testing will not be required.

The results of Tier I environmental toxicology tests will also determine the need for, and extent of environmental fate testing in Tier II. If required in Tier II, fate studies will only apply to MPCAs that are non-indigenous to the ecozone(s) of intended use, as defined in Section 5, Part 8, *Environmental Fate*, of these guidelines.

The environmental hazard of a non-indigenous MPCA may be considered a concern if:

- the Tier II environmental toxicology studies indicate a wide host range or significant effects are noted on environmentally or economically important non-target organisms; and
- the environmental fate studies (Part 8) indicate that the MPCA is persistent or dispersive.

(*iii*) **Tier III:** Tier III testing will be required for non-indigenous MPCAs that produce significant adverse effects on non-target test organisms in Tier II.

Indigenous MPCAs are exempt from testing at Tier III even if adverse effects are noted on test organisms in Tier II. If Tier III testing is required for non-indigenous species, a reduced but representative number of the test species adversely affected by the MPCA in Tier II should be selected for testing.

There are two elements to the testing requirement in Tier III. The first is a definitive toxicity test in which the dose-response relationship, e.g., LC_{50}, between the MPCA and test organism is determined. The second is a chronic or life-cycle study in which the long-term effect(s) of low-level exposure to the MPCA is assessed.

In definitive toxicity tests, the endpoint must reflect the pesticidal activity of the MPCA, i.e., if the MPCA produces a toxin and has little or no infectivity, then the appropriate endpoint would be mortality. If, however, the mode of action is pathogenicity, then a more appropriate endpoint would be overt symptomatology. As appropriate to the mode of action of the MPCA, the Tier III test data should establish the LD_{50} or LC_{50}, defined as the dose or concentration required to kill 50% of the test organisms, or the ED_{50} or EC_{50}, defined as the dose or concentration necessary to produce overt symptomatology in 50% of the test organisms. The PMRA recognizes that it would be difficult to establish specific LD_{50}, LC_{50}, ED_{50} or EC_{50} values for most MPCAs whose mode of action is pathogenicity, because test data are not likely to follow a dose-response relationship that is typical of chemical pest control agents. Therefore, data that establish an LD_{50}, LC_{50}, ED_{50} or EC_{50} that is greater than the challenge concentration in Tier II would often be adequate for risk assessment purposes of pathogenic MPCAs. For MPCAs that produce pesticidal toxins, however, a typical dose-response relationship is expected, thereby allowing for a definitive LD_{50}, LC_{50}, ED_{50} or EC_{50} with confidence limits to be established.

In chronic toxicity testing, the effect of the MPCA throughout, or at various stages of the life cycle of the non-target test organism as well as the ability of the non-target test organism to transmit the MPCA to its progeny is established.

Chronic toxicity testing of some non-target species may require a different route of exposure than the routes employed in Tier I and Tier II testing. For chronic testing of birds for example, a dietary route of exposure would be appropriate. Exposure concentrations should also be lower than those used in Tier II testing. For most chronic tests, the challenge concentration should be equivalent to the residue level expected immediately following a direct application of the MPCA at the maximum label rate.

(*iv*) **Tier IV:** Tier IV simulated or actual field testing of indigenous and non-indigenous MPCAs is required when adverse effects are produced on non-target organisms in Tier II. Tier IV studies are to involve small-scale terrestrial or aquatic field testing to determine whether adverse effects can be produced on susceptible non-target organisms under operational conditions of use. Testing with the EP is required and exposure concentrations should be equivalent to the maximum recommended application rate proposed for the product label.

(f) Non-Target Organism Testing

Part 9.1 Summary

Part 9.2 Birds

Non-target avian species are likely to be exposed to the MPCA most commonly through the oral route via feeding of infected food, e.g., insects, or through the respiratory tract via spray drift or aerosolization. Therefore, an avian oral test and an avian pulmonary test will be required for all microbial pesticides. For Tiers I, II and III (definitive toxicity) tests, the MPCA is to be administered to the gut by oral gavage or intubation and to the respiratory tract by intranasal or intratracheal instillation. The dietary route of administration was considered for Tiers I and II tests, but was found to not conform with the maximum hazard testing approach adopted by the PMRA. The diet, however, is considered to be an appropriate route of administration for Tier III (life cycle) and Tier IV tests.

Depending on the physical properties of the TGAI being tested, aerosolization may be used as an alternate pulmonary exposure route to intranasal or intratracheal instillation. If aerosolization is chosen by the applicant as a route of exposure, a written justification for choosing this method should be submitted to the PMRA.

An injection, i.e., intravenous or intraperitoneal, test may be substituted altogether for the pulmonary test if the microbial dosing preparation is sufficiently free from exogenous protein and other contaminating substances that would otherwise confound the test. Although this route is environmentally unrealistic, it provides a maximum hazard challenge by bypassing the bird’s primary defence mechanisms.

(*i*) Test Species

Testing should be conducted on one avian species, preferably bobwhite quail (*Colinus virginianus*) or mallard duck (*Anas platyrhynchos*), as they are ecologically significant, have proven to be good laboratory test species and are appropriate for acute, subacute and chronic testing. Other avian species, however, would also be acceptable for testing, especially altricial species. If a species other than bobwhite quail or mallard duck is selected for testing, a justification must be supplied based on increased susceptibility to the MPCA or ecological considerations that preclude the use of recommended species.

For Tiers I, II and III (definitive LD_{50}) environmental toxicology tests, young birds approximately 14 days of age at the beginning of the test should be used. Within a given test, all birds should be as near the same age as possible.

(*ii*) Maximum Challenge Concentration (MCC)

For Tier I tests, the MCC should be a function of the concentration of the MPCA in the TGAI and the route of administration.

C	Oral	=	MPCA Concentration in TGAI (units MPCA/mL) × 5.0 mL/kg Body Weight × Weight of Bird (kg)
C	Pulmonary	=	MPCA Concentration in TGAI (units MPCA/mL) × 0.2 mL/kg Bird Body Weight × Weight of Bird (kg)
C	Intravenous	=	MPCA Concentration in TGAI (units MPCA/mL) × 0.5 mL/kg Bird Body Weight × Weight of Bird (kg)
C	Intraperitoneal	=	MPCA Concentration in TGAI (units MPCA/mL) × 2.0 mL/kg Bird Body Weight × Weight of Bird (kg)

A justification should be provided to support any reduction in the maximum dose tested in Tier I.

For MPCAs that are expected to increase significantly in the environment following an application, e.g., viruses in insects, the oral dose administered should be no less than the highest concentration possible in the field, e.g., equivalent to the numbers in maximally infected insects.

(*iii*) Testing Requirements

Part 9.2.1 Avian Oral

Tier I acute oral tests should consist of the MCC administered by gavage to each test bird daily for five successive days.

Part 9.2.2 Avian Pulmonary, Inhalation or Injection

Tier I acute pulmonary tests should consist of intratracheal instillation of the MCC daily for five successive days. Pulmonary data conducted using the alternative inhalation, i.e., aerosolization, method should also expose birds to the MCC daily for five consecutive days. If the injection route of administration is substituted for the pulmonary test, the test should consist of the MCC administered as a single dose by intraperitoneal injection for fungi and protozoa, or by intravenous injection for viruses and bacteria.

For Tier II acute oral and acute pulmonary tests, the birds should be dosed once at the MCC. The relevancy of effects produced by the intraperitoneal or intravenous injection route of administration will be considered on a case-by-case basis and, thus, the applicant may not need to repeat these tests in Tier II, particularly if the dosing regime is the same as in Tier I.

If significant adverse effects are not observed in Tier I tests, additional testing at higher tiers is not normally required. However, if it is determined that there is a potential hazard to birds despite the negative Tier I results, additional testing may be required. As a MPCA that produces significant adverse effects on birds in Tier II tests could pose an unacceptable risk, submission of Tier III and Tier IV tests will not routinely be required in an initial registration submission, but may be requested following the initial review of Tier I and Tier II test results.

Part 9.3 Wild Mammals

The toxicology data required in Section 5, Part 4, *Human Health and Safety Testing*, of these guidelines to evaluate hazard to human health and safety are usually adequate to indicate hazard to wild mammals. Under certain conditions, however, these data may not be sufficient to assess the potential hazard to wild mammals. For example, if there is considerable variation in the sensitivity of different mammalian species to the effects of the MPCA, and if wild mammals are expected to be heavily exposed to the MPCA under operational conditions of use, then additional testing may be required.

If testing with wild mammals is required, tests should be performed on representative species from the ecozone(s) of intended use that are most likely to be affected by the use pattern of the MPCA. Test animals may be reared in pens or captured in the wild and must be phenotypically indistinguishable from wild mammals. The actual form of the MPCA to be tested is described in Section 5, Part 4, *Human Health and Safety Testing*, of this document. In addition, any substances used to enhance virulence or toxicity should be tested along with the test substance.

Theoretically, there is a potential for microbial pesticides to disrupt the normal function of rumen bacteria in ungulates. Tests on rumen function in wild ruminant mammals may be required in cases where such effects are considered likely or if effects are reported in domestic animals.

With respect to appropriate Tier I dosing concentrations, route(s) of exposure and controls, applicants should refer to instructions in Section 5, Part 4, *Human Health and Safety Testing*, of these guidelines. If higher tier testing is required, the testing approach should be similar to that for birds, adapted appropriately for mammalian test methods. In addition to the information specified in Section 5, Part 4, *Human Health and Safety Testing*, reports should contain the same information required for the avian oral and avian pulmonary tests, adapted appropriately for mammalian test procedures.

If no significant adverse effects are observed in Tier I, i.e., Part 4, *Human Health and Safety Testing* tests, additional testing at higher tiers will not ordinarily be required. If it is determined, however, that there is a potential hazard to wild mammals despite the negative results obtained from Part 4, *Human Health and Safety Testing*, additional testing may be required. As an MPCA that produces significant adverse effects on mammals in Tier II tests could pose an unacceptable risk, submission of Tier III and Tier IV tests will not routinely be required in an initial registration submission, but may be requested following the initial review of Tier I and Tier II test results. For MPCAs or EPs that demonstrate a potential to adversely affect mammals in the wild, applicants should consult with the PMRA prior to initiation of higher tier tests.

Part 9.4 Fish

In testing the effects of the MPCA on non-target species of fish, two routes of exposure should be considered: (1) suspension in the test water, i.e., aquatic exposure; and/or (2) diet, either in the form of diseased target pests, i.e., hosts, or incorporation of the MPCA in standard fish feed. Aquatic exposure would simulate the type of natural exposure expected immediately after direct application and would also simulate the route of exposure that many known pathogens use to infect fish. Dietary exposure would also simulate certain natural conditions and is perhaps the most important means of exposure if the normal hosts of the MPCA are a major component of fish diet. Dietary exposure using diseased hosts as feed would further increase the possibility of exposing the test fish to a different life stage of the microorganism than may be present in the EP or in amended artificial feed stock.

For Tiers I, II and III (definitive LC_{50}, EC_{50}) laboratory testing, actively feeding juvenile fish, three to six months old, should be treated rather than very young, not yet actively feeding, spawning, or recently spent fish. Testing of other adult life stages should also be avoided because adults have the potential to become carriers of some diseases and adult fish are not as susceptible to pathogens as juvenile fish. All test fish should weigh between 0.5 and 5.0 grams and be from the same year class. The length of the longest fish should be no more than twice that of the shortest fish.

(*i*) Test Species

Part 9.4.1 Freshwater Fish

Testing should be performed on one cold water fish species, preferably rainbow trout (*Oncorhynchus mykiss*) or a species of salmon such as Chinook (*Oncorhynchus tshawytscha*), coho (*Oncorhynchus kisutch*) or Atlantic (*Salmo salar*). If rainbow trout is selected as the test species, applicants should be aware that if adverse effects are noted in Tier I tests, testing will also be required on a salmon species.

Part 9.4.2 Estuarine or Marine Fish

In cases where estuarine or marine waters are likely to be impacted, the MPCA should also be tested for salinity tolerance. If it is determined that the MPCA is capable of surviving in water with a salinity of 10 parts per thousand or more, the tests should include, in addition to the freshwater or salmon species, one estuarine or marine fish species such as the sheepshead minnow (*Cyprinodon variegatus*).

If the MPCA is intended to control nuisance fish, e.g., sea lamprey or gizzard shad, effects testing on other non-target fish species will be required. Selection of appropriate numbers and species of fish should be based on the centrifugal taxonomic approach described above in *Selection of Non-Target Organisms* and take into account species that occur in drainage basins in the areas, i.e., ecozones, of intended use. Consideration should also be given to species that are likely to prey upon or scavenge the diseased target host.

(*ii*) Maximum Challenge Concentration (MCC)

Treatment concentrations must be related to the number of microorganisms to which fish may be exposed under actual use conditions. The highest feasible concentrations should be used in all exposures.

For aqueous exposure data, fish should be exposed to an MCC of at least:

- 10^6 viable units of the MPCA per millilitre of water; or
- 1000 times the expected environmental concentration of the MPCA, immediately following a direct application at the maximum label rate to a 15-cm layer of water, whichever is greater or achievable.

The use of such a high concentration may be limited by its adverse effect on water quality such as oxygen depletion and production of metabolic byproducts by the microorganisms. It is recommended, therefore, that the test water be renewed at a sufficient rate to maintain water quality and concentration of the MPCA.

For dietary data, the MCC in the feed, i.e., active units of the MPCA per gram of feed, should be equivalent to the maximum concentration found in the target, i.e., active units of the MPCA per gram of target. Alternatively, fish can be fed the target organisms that have been maximally infected with the MPCA. If the latter diet is chosen, additional data may be required on the effects of the additives in the EP, if these additives are intended to enhance virulence or toxicity of the MPCA.

(*iii*) Testing Requirements

For Tier I, test fish should be exposed to the MCC of the MPCA for the duration of the study period. During testing, fish should be periodically examined for the incidence of any adverse effects. If any adverse effects are observed, then a period of recovery should be instituted immediately on half of the tank replicates to determine whether the effects are reversible. The dissemination, replication, and survival of the MPCA should also be determined in representative tissues, organs, and fluids.

For tests on marine fish, the salinity of the test water should be maintained between 30 and 35 parts/thousand. For tests on estuarine species, the salinity should be between 10 and 20 parts/thousand.

If Tier II environmental toxicology testing is indicated, then the route(s) of exposure that produced the greatest impact on test organisms in Tier I should be tested. For Tier II aqueous exposure data, test fish should be exposed for five days to the MCC of the MPCA. For Tier II dietary data, test fish should be fed the MCC for five days. All fish should be monitored for any adverse effects observed in Tier I data. Regardless of the exposure route chosen for Tier II testing, the test fish should be monitored for an additional 25 days following dosing or until adverse effects are observed.

Tier III life cycle testing is required for non-indigenous MPCAs that produce significant adverse effects on non-target organisms in Tier II. In addition to life-cycle tests, definitive toxicity testing to establish the LC_{50} or EC_{50} of the MPCA is also required in Tier III. Indigenous MPCAs are exempt from Tier III testing even if adverse effects are observed at the Tier II level.

For both indigenous and non-indigenous MPCAs, simulated or actual Tier IV field testing may be necessary to assess the hazard of the MPCA under actual use conditions if Tier II environmental toxicology test results indicate a potential for adverse effects on fish.

Part 9.5 Arthropods

Test arthropods should be exposed to the MPCA in a manner consistent with the route of exposure, mode of action, and greatest degree of susceptibility under natural environmental conditions. The route of exposure selected for testing is based mainly on whether the non-target arthropod is terrestrial or aquatic.

Part 9.5.1 Terrestrial Arthropods

The MPCA should be administered:

- topically;
- in the diet; or
- as a combination of these two routes of exposure.

Part 9.5.2 Aquatic Arthropods

The MPCA should be administered:

- as a suspension in the water, i.e., aquatic exposure;
- in the diet; or
- as a combination of these two routes of exposure.

Periodic monitoring of the MPCA in the test water is required to determine the concentration and infectivity potential of the MPCA to which the non-target arthropod is exposed.

(*i*) Test Species

Non-target arthropod species selected for testing should be representative of groups that will be exposed to the MPCA under actual conditions of use, and that have some important relationship with the target pest species. The applicant should refer to Section 5, Part 9, d, *ii) Selection of Non-Target Organisms* for guidance in selecting test species. Particular consideration should be given to arthropods established in the ecozone(s) of intended use, and to those "beneficial" species with broad environmental or economic importance, such as bees, *Apis mellifera* (L.) (Hymenoptera), insects used for biological control, e.g., *Apanteles* spp. (Hymenoptera), predatory orchard mites, e.g., *Neoseilus fallacis* (Garman) (Acari), *Phytoseiulus persimilis* (Acari), etc.

As a result of the difficulties in culturing some arthropods and the scarcity of established testing protocols, some taxa have been studied to a greater extent than others, e.g., Noctuidae vs. Arctiidae in the Order Lepidoptera, Lepidoptera vs. Neuroptera. Consequently, some adjustment in species selection may be necessary to account for these differences in protocol availability. Nevertheless, the non-target arthropods used in the toxicology tests should be those considered to be the most susceptible to injurious effects caused by the MPCA.

For MPCAs applied in terrestrial use patterns, where direct aquatic exposure is not anticipated, it is recommended that, wherever possible, representatives of the terrestrial non-target arthropod taxa listed in Appendix XI, *Suggested Taxa for Selection of Non-target Arthropods*, be selected for testing. Each of the taxa listed contains environmentally or economically important species that may be obtained from Canadian arthropod culture collections.

For MPCAs applied in aquatic use patterns, or applied in terrestrial use patterns where aquatic exposure is anticipated, testing on aquatic non-target arthropods is required. Appendix XI, *Suggested Taxa for Selection of Non-Target Arthropods*, lists the freshwater and estuarine or marine aquatic arthropod groups from which test species should be selected.

(*ii*) Maximum Challenge Concentration (MCC)

For topical exposure tests, non-target arthropods should be exposed to a concentration of the MPCA that is equivalent to 100 times the maximum rate of application.

For exposure tests that require an application directly to soil or water, the arthropods should be exposed to an MCC of at least:

- 10^6 active units of the MPCA per gram of soil or water; or
- 1000 times the expected environmental concentration of the MPCA, immediately following a direct application at the maximum label rate to a 15-cm layer of soil or water, whichever is greater or achievable.

The use of such a high concentration in aqueous systems may be limited by its adverse effect on water quality such as oxygen depletion and production of metabolic byproducts by the microorganisms. It is recommended, therefore, that the test water be renewed at a sufficient rate to maintain water quality and concentration of the MPCA.

For artificial dietary data, the MCC, i.e., active units of the MPCA per gram of feed, should be equivalent to the maximum concentration found in the target, i.e., active units of the MPCA per gram of target. Alternatively, arthropods can be fed the target organisms that have been maximally infected with the MPCA. In cases where it may be difficult to determine the maximum concentration in the target, arthropods can be fed a diet treated with an application of the MPCA equivalent to 100 times the maximum label rate. It should be noted that in cases where an infected target is chosen as the dietary route of exposure, additional data may be required on the effects of the additives in the EP, if these additives are intended to enhance virulence or toxicity of the MPCA.

(*iii*) Testing Requirements

For Tier I topical exposure tests, the non-target arthropod should be treated with the MCC for five successive days and then observed for an additional 16 days. For Tier I exposure or dietary tests, non-target arthropods should be exposed to, or fed the MCC of the MPCA or EP for at least 21 days, or until mortality in the control group increases to a significant level.

For Tier II environmental toxicology tests, arthropod species adversely affected in Tier I should be exposed to, or fed the MCC of the MPCA for five days and then observed for the equivalent period of time required for manifestation of adverse effects in Tier I tests. For Tier II topical exposure tests, susceptible arthropod species tested in Tier I should be treated once with an application of the MPCA equivalent to the maximum rate proposed on the product label.

Although Tier III life cycle testing may be triggered by the results of Tier II acute data, the PMRA recognizes that testing beyond the acute toxicity level may not be possible for some arthropod species. If Tier III testing is triggered, definitive toxicity testing to establish the LC_{50} or EC_{50} of the MPCA will also be required. Indigenous MPCAs are exempt from Tier III testing, even if adverse effects are observed at the Tier II level.

For indigenous and non-indigenous MPCAs, simulated or actual Tier IV field testing of susceptible non-target arthropods may be necessary to assess the hazard of the MPCA under actual use conditions if Tier II environmental toxicology test results indicate a potential for adverse effects on arthropods.

Part 9.6 Non-Arthropod Invertebrates

If the MPCA is intended to control invertebrates, such as annelids, e.g., *Lumbricus terrestris*, or molluscs, then effects testing on non-target non-arthropod invertebrates is required. In selecting appropriate non-arthropod invertebrates for effects testing with the MPCA, applicants should follow the centrifugal taxonomic approach as outlined in Section 5, Part 9, d, *ii) Selection of Non-Target Test Organisms*. Applicants should also follow the tier testing approach outlined above for arthropods to determine appropriate tests for non-arthropod invertebrates.

If the MPCA is not intended to control non-arthropod invertebrates, but may be closely related to a known pathogen to this group of organisms, then the MPCA should also be tested on potentially susceptible non-arthropod invertebrates that may be exposed during operational conditions of use. If the MPCA does not resemble any known or suspected pathogen to non-arthropod invertebrates, effects testing may still be required if exposure is a concern.

Part 9.7 Microorganisms

For most MPCAs, effects testing on non-target microorganisms will not be required. However, the PMRA has determined that in cases where the biology, ecology, and proposed use pattern(s) of the MPCA indicate a potential for adverse effects on environmentally or economically important microbial species or microbiologically-mediated biogeochemical processes, effects testing on these species or processes may be required. Testing of non-target microorganisms or processes will mainly be reserved for MPCAs intended to control pest microorganisms or processes. The requirement for microorganism testing is determined by the PMRA on a case-by-case basis.

If testing is required, a single route of exposure should be adequate to assess the effects of the MPCA on non-target microorganisms or microbial processes. In keeping with the maximum hazard route of exposure described for other non-target organisms, the MPCA should be administered directly to the environment in which the non-target microbial population or community is expected to occur.

(*i*) Test Species

Although the ecology and proposed use pattern will dictate the established species or processes most likely to be impacted by the MPCA, in most cases it will be difficult to ascertain the specific microorganisms, in the ecozone of application, that may be affected by the MPCA. Therefore, it is recommended that tests on non-target microorganisms be undertaken using a complex "natural" microbial ecosystem, i.e., microcosm, relevant to the area of application. Microcosm data should be used to examine the effects on relevant microbial biogeochemical processes such as carbon dioxide evolution, nitrogen transformations, cellulose degradation, etc., rather than changes, qualitative or quantitative, in the microbial community.

(*ii*) Maximum Challenge Concentration (MCC)

The non-target microbial population or community should be exposed to an MCC of at least:

- 10^6 active units of the MPCA per gram of soil or water; or
- 1000 times the expected environmental concentration of the MPCA, immediately following a direct application at the maximum label rate to a 15-cm layer of soil or water, whichever is greater or achievable.

(*iii*) Testing Requirements

Tier I testing should involve controlled laboratory tests under conditions that approximate those in the environment and pesticide ecozone(s) of intended use. In these data, the MCC of the MPCA should be used and emphasis should be placed on examining adverse effects on microbiologically-mediated biogeochemical

cycles. Observation periods are difficult to specify when testing the effects of MPCAs on non-target microorganisms or processes because they are highly dependent on the study endpoint. As a general rule, however, the observation period should be a minimum of 28 days following the application of the MPCA. If after 28 days, adverse effects deviate from the untreated control by more than 15%, sampling should be continued, in appropriate intervals, until differences are no longer evident, or a maximum of 100 days have elapsed from study initiation.

To aid in data interpretation, microcosm studies, Part 8, *Environmental Fate*, if required, should be carried out under analogous experimental conditions as the Tier I environmental toxicology data.

Testing beyond the Tier I level is not expected for most MPCAs. If results of Tier I data, however, indicate long-term and irreversible adverse effects on non-target microorganisms or processes of environmental or economic importance, then testing at higher tiers may be warranted. If testing at higher tier levels is required, the applicant should consult with the PMRA to identify appropriate tests and test methods for the MPCA in question.

Part 9.8 Plants

MPCAs intended to control pest plants, i.e., herbicidal MPCAs, and any EPs that may contain phytotoxic or phytopathogenic MPCAs, must be tested on non-target plants to determine their host range. MPCAs that are taxonomically similar to known plant pathogens with very narrow host ranges may only need to be tested on a limited number of potentially susceptible plant species. MPCAs that are similar to a wide range of known plant pathogens, however, may require testing on a greater number of plant species to determine its complete plant host range. Knowledge of the mode of action may also assist in determining the extent of testing needed for potential plant pathogens.

MPCAs not intended to control plants, i.e., non-herbicidal MPCAs, should also be tested for potential adverse effects on those non-target plants expected to be contacted during operational conditions of use. MPCAs that do not resemble any known plant pathogen would not be expected to have phytopathogenic properties and, therefore, would require little, if any, plant testing.

Test plants should be exposed to the MPCA or EP by whatever route of exposure would be expected by the proposed use pattern. This route of exposure should be supplemented by other routes of exposure if indicated by the transmission of typical pathogens of the test plant or for herbicidal MPCAs, if indicated by the route of transmission of similar plant pathogens. In some cases, wounding of plants or simulation of actual insect vectors may be appropriate. In other cases, seed treatment, root or soil application, foliar spraying, or direct application to water might be the most appropriate method.

(*i*) Test Species

Selection of test plants should take into account such factors as the purpose of the MPCA, i.e., whether it is intended to control plants; the pesticide ecozone(s) of intended use; the likelihood of exposure to the MPCA, as determined by the use pattern and routes of dissemination in the environment; and the phylogenetic proximity of the test species to target pest species. For additional guidance in selecting appropriate non-target plant species, refer to Section 5, Part 9, d, *ii) Selection of Non-Target Organisms*. Applicants should also endeavour to test some weed and wild plant species.

Selection of non-target plant species depends on whether the intended use pattern of the MPCA is terrestrial or aquatic.

With the exception of forestry applications, MPCAs intended for terrestrial uses where direct aquatic exposure in not anticipated or the MPCA is not expected to survive in aquatic environments, effects testing on aquatic non-target plants will not ordinarily be required. Conversely, if an MPCA is intended to control an aquatic plant, data on the effects of the MPCA on non-target terrestrial plants will not ordinarily be required unless it can be demonstrated that non-target terrestrial plants would be exposed to the MPCA, e.g., via spray drift from aerial applications.

Part 9.8.1 Terrestrial Plants

For MPCAs intended for terrestrial use, where direct aquatic exposure is not anticipated, test species should be selected from up to 12 representative terrestrial plant families listed in Appendix XII, *Suggested Taxa for Selection of Non-Target Plant Species*. Since the characteristics of the MPCA will ultimately determine the plants to be tested, the plant families in Appendix XII are provided only as a guide. These families were selected for their environmental or economic importance in Canada.

For MPCAs intended for forestry use where direct aquatic exposure is anticipated, species of Pinaceae and Silicic, and some species of aquatic plants should also be selected for testing.

For non-herbicidal MPCAs that are taxonomically related to known phytopathogenic microorganisms, plant species susceptible to the known pathogens should be selected as a starting point to assess the phytopathogenicity of the MPCA. If pathogenic effects are noted in any of these species, further testing utilizing the centrifugal taxonomic approach would be required with the affected plant species as the new starting point.

Part 9.8.2 Aquatic Plants

For MPCAs intended for aquatic use or for terrestrial use where aquatic exposure is anticipated, test species should be selected from up to six representative aquatic plant families listed in Appendix XII, *Selected Taxa for Selection of Non-Target Plant Species*. The centrifugal taxonomic approach should be used to select species for testing against MPCAs intended to control aquatic plants.

Additional effects testing is also required on at least one representative species from each of the algal families Chlorophyceae (green), Cyanophyceae (blue-green), and Bacillariophyceae (diatom).

Testing on marine plant species will not ordinarily be required unless, as a result of the use pattern, a potential exists for exposure of marine plants.

(*ii*) Maximum Challenge Concentration

In most cases, the MCC should be equivalent to spraying the plant to runoff with a concentration of the MPCA that is equivalent to the maximum rate of application proposed on the product label. For seed treatments, seeds should be thoroughly drenched with the MPCA. For applications directly to soil or water, test plants should be exposed to at least:

- 10^6 active units of the MPCA per gram of soil or water; or
- 1000 times the expected environmental concentration of the MPCA, immediately following a direct application at the maximum label rate to a 15-cm layer of soil or water, whichever is greater or achievable.

(*iii*) Testing Requirements

For Tier I testing, non-target plants should be treated with the MCC of the MPCA at the time of most likely susceptibility or at the normal stage of maturity that the plants would be exposed to the MPCA under actual conditions of use. When the optimum conditions for penetration, infection and disease development are known or suspected, it is important, particularly for herbicidal MPCAs, to simulate these conditions rather than those known to be optimum for plant growth and development. In many cases, however, the optimum environment may be similar. Test plants should be observed regularly until normal harvest or death or, as in the case of perennials, at periodic intervals for at least the time required to adversely affect the target plant pest. If no adverse effects are evident after these observation periods, plant tissues should be analysed for the presence of the MPCA using sensitive, specific methods as asymptomatic plants may serve as sites for proliferation and survival of the MPCA in the environment.

The results of Tier I tests will be used in conjunction with available information on use pattern, host range and other similar factors to assess the potential for adverse effects on non-target plants. Adverse effects observed on senescent leaves would be considered of minor importance and would not warrant Tier II testing; however, the plants should be observed during Tier I testing to determine if the infection or effects spread to viable tissues or result in a decrease in plant vigour.

For Tier II environmental toxicology testing, data should be conducted on environmentally or economically important non-target plant species for which an infection or other adverse effect was observed in Tier I. For certain terrestrial non-target plants, testing of both the germinating seed, i.e., seed emergence and root elongation testing, and young vegetative growth, i.e., vegetative growth and vigour testing, will be required. Plants should be tested with a concentration of the MPCA or EP that is equivalent to the maximum rate of application proposed on the product label.

Tier III testing will be required for non-indigenous MPCAs if the results of Tier II environmental toxicology tests indicate that non-target plants may be adversely affected by the MPCA under operational conditions of use. If required, Tier III tests should be carried out in the laboratory. Data on the fate of the non-indigenous MPCA in affected plant tissues may be also required with Tier III testing.

Tier IV testing should be conducted on small-scale field plots with the EP applied at the maximum label rate and under conditions that are optimal for disease development in plants. Only susceptible non-target plant species identified in Tier II tests should be tested in Tier IV. Control treatments, including untreated target and non-target plants as well as treated target plants are essential in Tier IV data. Tier IV data may be combined with small-scale environmental fate field trials and efficacy data. Tier IV testing of aquatic non-target plants is not required for small-scale terrestrial field trials. However, since the effects on non-target aquatic plant species may be unknown, the dissemination of the MPCA must be restricted to prevent possible adverse effects on non-target aquatic plants.

Part 10 Value Assessment (including Efficacy)

(a) Introduction

Consistent with the requirements of the PCPA and Regulations and the mandate of the PMRA, assessment of the value of the EP in the context of its proposed uses is required.

For the purposes of this guideline, value comprises objective information regarding the following:

(*i*) the nature and economics of the pest or disease problem in Canada;
(*ii*) EP performance, according to prescribed label conditions and claims;
(*iii*) current management tools: status, benefits, problems; and
(*iv*) the contribution of the EP to risk reduction and sustainable pest management in the specific crop or resource production system.

The purpose of value assessment is to promote balanced regulatory decisions that incorporate consideration of the potential unique or long term benefits of the EP to sustainable crop and pest management or risk reduction with possible drawbacks or disadvantages. It is anticipated that EPs will demonstrate special characteristics that adapt well to integrated management programs or may exhibit desirable safety features. Formal documentation of value is an important component of risk management decision making, especially in those difficult situations where the scientific data base supporting the proposed registration may not be ideal.

(b) Product Performance

Product performance is defined as the ability of the EP to fulfill the claims made on the proposed label. It includes the nature and extent of control or management of the pest or disease problem and also considers beneficial or adverse effects on the host crop and the crop production system.

Product performance data should provide information regarding the MPCA's pest host range, time to mortality and the minimum dosage required to achieve the desired or claimed standard of performance according to the specifics of the proposed use scenario. Such information is developed to support label performance claims and use recommendations, and is also important to non-target organism safety assessment. Performance data will also serve to confirm, where applicable, the utility of the EP in the development of sustainable pest management practices, including integrated management strategies, resistance management and risk reduction.

(c) General Provisions

Where practicable, performance data packages should be based on original Canadian data. Foreign data collected under conditions comparable to intended Canadian use situations may be used to support Canadian registrations. Proponents of such MPCAs should consult with the PMRA to discuss appropriate performance criteria and test specifics.

For outdoor uses, e.g., forestry, agriculture, field data from relevant regions in the U.S. and other countries are acceptable, provided that the environmental conditions, pest behaviour and cultural practices are comparable. For uses under confined or controlled-environment conditions, e.g., greenhouses, interior plant scapes, foreign data will normally be acceptable, provided that the test conditions and crop management practices are demonstrably similar to those found in Canada.

Laboratory and field data to support label performance claims, including use recommendations, restrictions and contraindications, i.e., optimum methods and timing of application, host or pest phenological requirements and constraints, population density, environmental factors, etc., are required. Proponents should consult Regulatory Directive DIR93-07*a*, *Guidelines for Efficacy Assessment of Chemical Pesticides*, for guidance regarding the principles of efficacy testing, including experimental design, performance criteria and analysis and reporting of data.

Specific guidance documents concerning performance testing of products intended for disease management, Regulatory Directive DIR96-01, *Guidelines for Efficacy Assessment of Fungicides, Bactericides and Nematicides*, and competing vegetation management, Regulatory Directive DIR93-07*b*, *Guidelines for Efficacy Assessment of Herbicides and Plant Growth Regulators*, should also be consulted, as applicable to the EP.

NOTE: *Although the referenced efficacy documents outline the approach to chemical pesticide testing, proponents are advised that the general principles described apply equally to performance testing of most EPs. A thorough understanding of these principles is essential to preparing an acceptable product performance data package.*

It is also noted that these are guidelines only; common sense and scientific judgement should be exercised in their use and application to microbial testing.

The majority of MPCAs in commercial use today are effective only when used in an inundative manner. As a consequence of constraints that may result from relatively moderate virulence or toxicity or limited field persistence, the achievement of pest management goals often requires one to several EP applications per season. For this reason, the fundamental principles of efficacy testing developed for traditional control products are considered to be applicable to many EPs. It is recognized that these principles may not be appropriate to the assessment of performance for MPCAs intended for use as inoculative or augmentative biocontrol measures.

(d) Evaluation Criteria and Procedures

The criteria and procedures used by the PMRA for evaluation of Part 10, *Value Assessment*, are outlined in Section 2.3 of the Regulatory Directive DIR93-07*a*, *Guidelines for Efficacy Assessment of Chemical Pesticides*. Regarding sources of information considered in the review, it is emphasized that it is the responsibility of the applicant to produce, collect, organize and submit all scientific information required to support the proposed registration. The PMRA may also use any relevant data available in addition to those submitted by the registrant. Only scientific evidence will be used in the assessment of the value of a product. Testimonials of individuals without documented evidence are of no value in performance evaluation.

(e) Principles and Data Requirements

Part 10.1 Summary

Part 10.2 Performance Assessment

Part 10.2.1 Laboratory and Growth Chamber Studies

For the purposes of this guideline, laboratory and growth chamber studies denote quantification, by appropriate means, of MPCA activity on potential target pests or hosts, e.g., preliminary range finding tests, preliminary target pest screening. These might include conventional diet or foliage feeding bioassays, single plant or tree exposure, in vitro antagonism data, growth chamber or greenhouse data, etc., as appropriate to the nature of the EP.

Laboratory studies should be designed to describe the intrinsic susceptibility, dose response behaviour, time to mortality, relative susceptibility of target pests or hosts, etc., as appropriate to the nature and activity profile of the MPCA. The relationship of challenge doses used in laboratory studies to the expected exposure concentration of the MPCA under actual use conditions should be discussed. Where relevant, laboratory studies should also address the susceptibility of various life stages of the proposed target pests or hosts.

For MPCAs that exhibit a direct, measurable toxic or lethal effect, scientifically sound laboratory studies designed to quantify the susceptibility, dose response behaviour and time to mortality on proposed target pests or hosts are normally required.

Laboratory data should also incorporate studies that describe the pest host spectrum of activity of the MPCA. These studies would normally be carried out at doses defined in relation to the LC_{50}, LC_{95}, LT_{50}, etc., of the most susceptible target pest. Alternatively, these data might also be generated using challenge doses based on the expected exposure concentration of the MPCA under actual use conditions.

The TGAI or FI may be used for laboratory bioassays, provided that any adjuvants or other constituents of the formulation deliberately added to enhance toxicity or virulence are tested in combination with the MPCA. Where more meaningful to the assessment of efficacy, the EP may also be used.

For new MPCAs, laboratory bioassay data will generally form the basis for preliminary target susceptibility screening and will contribute to evaluation of field performance. It is recognized that host or pest phenology, target pest behaviour and environmental constraints profoundly influence activity of MPCAs in the field. Where possible, information or data that describes the relationship of in vitro bioactivity, i.e., intrinsic susceptibility, to that expected or observed in the field should be provided.

These data, in conjunction with information from Part 9, *Environmental Toxicology*, will be used in the assessment of potential environmental risk and are also important to evaluation of claims regarding risk reduction and contribution to sustainable pest management.

For use expansions to closely related targets, laboratory or greenhouse data may, in some instances, serve to reduce or eliminate the need for field efficacy studies. In this case, it is recommended that testing be carried out using the EP rather than the technical material. Proponents should consult the PMRA prior to testing when seeking label amendments.

Where laboratory bioassay data are not deemed appropriate or meaningful to the assessment of value, the proponent may request a waiver of laboratory studies with submission of a supporting scientific rationale.

Part 10.2.2 Field Studies

For the purpose of this guideline, field studies refer to testing of the proposed EP under conditions equivalent to and according to the use patterns proposed on the end-use label.

Experimental field data should be developed and reported according to the principles outlined below and in the companion chemical efficacy guidelines. In general, the data should demonstrate in a scientifically acceptable manner that when used according to label recommendations, the EP will provide a meaningful benefit to the user.

(a) Performance Criteria

Since MPCAs constitute a diverse spectrum of biological entities, they potentially include all types of pest management products. Field performance criteria and the specifics of experimental design will thus vary, depending on the biological characteristics of the MPCA, the nature of the pest management problem and the goals of treatment.

It is recognized that MPCAs may demonstrate performance benefits in a specific crop or resource production system in various ways, both direct and indirect. Examples include direct mortality to the target that results in control or suppression of pest populations to defined levels; plant growth promotion and prevention of disease establishment; induction of host plant resistance; and sublethal effects on pest targets that enhance natural biocontrol or increase susceptibility to conventional control products. Criteria to measure and define performance may thus sometimes differ substantially from those applied to conventional chemicals. In light of this, it is essential that the specific EP performance criteria employed be well defined and that the goal(s) of treatment be clearly stated.

For convenience, the key principles that should be observed in the design and execution of EP field efficacy trials are summarized below. Proponents should also consult Section 2, Regulatory Directive DIR93-07*a*, *Guidelines for Efficacy Assessment of Chemical Pesticides*, for a more detailed explanation.

(*i*) Performance testing should be conducted using the EP formulation proposed for registration. Subsequent minor formulation changes will be acceptable, provided that scientific rationale and appropriate bridging data to support equivalent performance are submitted.

(*ii*) In cases where the EP is not yet in full scale commercial production, a time-limited registration may be considered, provided that performance data on pilot scale material supports the value of the EP and that all other data requirements are met. Subsequent full registration will require confirmation of performance using commercial scale material.

(*iii*) The biological activity and potency of each specific lot of EP tested should be confirmed, as per methods reported in Part 2, *Product Characterization and Analysis*, and the results included in the efficacy report. The biological activity of the test substance at the time of testing should be representative of the product specifications and guarantee.

(*iv*) Where biological activity of the EP requires ingestion by the target pest, performance trials should incorporate data to determine optimum conditions and methods of application necessary to ensure adequate deposit to the pest feeding site(s). Valid deposit measurements may sometimes be necessary to aid in interpretation of performance data, e.g., aerial application.

(*v*) Performance trials should be carried out under the conditions of application and at rates or doses proposed on the label. In general, at least one range finding field study demonstrating the lowest effective rate is required.

(*vi*) Each pest or disease and host combination on the proposed product label should be assessed separately. The use of surrogate pest species or hosts may be considered for some EPs, in lieu of data on each combination, provided that the MPCA mode of action and target specificity, the pest or disease behaviour and the host phenology warrant this approach. An adequate scientific rationale and laboratory bioassay, or equivalent data to support surrogate claims are required.

(*vii*) As a general rule, a minimum of three studies or trials, each with internal replicates, should be submitted. For outdoor uses, the studies should be conducted in the major geographical regions where the product is intended to be used. The studies should also take into account different population pressures and any environmental conditions that significantly affect EP activity. Artificially infested plots may be used where insufficient pest or disease pressure exists.

(*viii*) Notwithstanding the point above, regional variability in climate, soil type, pest behaviour, host phenology, cultural practices, management goals, etc., may necessitate supplementary trials to adequately demonstrate performance claims under all conditions of application and in all sites and areas of intended use. Proponents may request waiver of some regional studies if:

- only a limited, regional registration is desired; or
- scientific rationale to support reduced regional testing are submitted.

(*ix*) Trials should include an untreated check or control as an indicator of pest or disease pressure and for comparison with treated areas. Positive controls, comprising comparison with reference products of known efficacy or normally accepted practice should also be included where possible.

(*x*) Performance measurements should include effects on the quality and where appropriate the yield of treated crops or effects on the quality of treated plant products.

(*xi*) Label recommendations regarding tank mixing with registered pesticides or use of the EP with adjuvants, stickers, spreaders, diluents, etc., should be supported by compatibility and performance data.

Part 10.3 Treatment Effects

During the course of field efficacy testing, compatibility and beneficial or adverse effects of EP treatment on pest management within the specific crop production system, should be evaluated. The following aspects of EP behaviour should be addressed, as appropriate to the nature of the EP and the use pattern.

Part 10.3.1 Phytotoxicity and Phytopathogenicity

Information on possible phytotoxic or phytopathogenic effects to target crops or to target plant products is a required component of performance evaluation. For those EPs containing MPCAs with potential for plant injury, i.e., known or suspected plant pathogens, scientifically sound laboratory and/or field experiments to demonstrate lack of MPCA injury to labelled and adjacent crops/hosts are required. For other EPs, crop tolerance should be monitored during performance trials. Significant adverse effects should be reported. Observations on unintended or undesirable side effects on succeeding crops or parts of treated plants used for propagating purposes, e.g., seeds, cuttings, runners, should be reported.

Part 10.3.2 Compatibility with Crop Protection and Management Practices

Part 10.3.2.1 Effects on MPCA Performance

Information on the efficacy and compatibility of the EP when used in conjunction with other crop protection measures, particularly chemical pesticides, is required. This is especially important in crop production situations where current management practices involve use of control measures to which the MPCA may be sensitive. In the latter case, laboratory and/or field data that assess compatibility of the MPCA should be submitted.

In some instances, cultural practices or other chemical inputs may also adversely affect EP performance. These and any other crop management practice that may negatively affect performance should be evaluated, where relevant to the nature of the EP and the production scenario.

Part 10.3.2.2 Effects of the EP

Claims regarding enhancement of, or compatibility with natural biocontrols or IPM strategies should be supported by appropriate laboratory or field evaluation data as relevant to the nature of the claim, the characteristics of the MPCA and the proposed use pattern.

Host spectrum data, i.e., Part 10.2, *Performance Assessment* (see Section 5, Part 10.2), and relevant non-target hazard testing data, i.e., Part 9, *Environmental Toxicology* (see Section 5, Part 9), may be used to support claims regarding comparability and potential value to natural biocontrol and IPM. Where relevant to the specific claim, it is recommended that field performance testing include observations regarding effects of treatment on natural biocontrols within the specific crop or system.

Part 10.4 Crop or Resource Production Benefits

To document the potential value of a new EP in terms of crop or resource production benefits and sustainable management considerations, objective information in the following areas is required. It is the responsibility of the proponent to provide detail sufficient to draw conclusions regarding the potential or actual benefits of the new EP.

New EPs may demonstrate crop or resource production benefits or contribute to the implementation of more sustainable management practices or risk reduction in a number of ways. For example, new EPs might:

- **(*i*)** enhance or complement more sustainable use of current management products by reduction of label rates or frequency of application required for conventional products;
- **(*ii*)** provide a viable alternative to traditional products wherein significant problems or concerns exist, e.g., pest resistance, safety issues;
- **(*iii*)** provide entirely novel approaches to pest management in situations where conventional tools do not exist or are considered unacceptable for a variety of reasons;
- **(*iv*)** suppress pest populations to levels sufficient to promote commercially acceptable efficacy of more sustainable, non-interventional management practices, e.g., crop rotation, physical barriers;
- **(*v*)** encourage implementation of pest management practices such as scouting, timing and application of pest control products based on appropriate action or economic thresholds;
- **(*vi*)** constitute essential, narrow spectrum tools that provide the foundation for development of IPM strategies; and
- **(*vii*)** enhance occupational or bystander safety in situations where significant exposure may be unavoidable, e.g., corn tasselling, indoor uses, aerial application in residential areas.

Part 10.4.1 Profile of the EP

This section should include a synopsis of relevant information that summarizes the performance benefits.

Part 10.4.2 Nature and Economics of the Pest or Disease Problem in Canada

The nature and economics of the pest or disease management problem in Canada should be outlined. Reference may also be made to adjacent regions of the U.S. where relevant. The information should be presented in the context of the proposed use pattern and anticipated impact of the EP on the problem.

Most EPs, because of their relatively limited pest host range, will constitute niche or minor use products in Canada. The discussion should include some indication of the projected use of the new EP and should consider the following elements:

(*i*) range of the pest or disease;
(*ii*) life cycle of the pest or disease in Canada;
(*iii*) crops (resource) affected;
(*iv*) qualitative and quantitative damage by the pest or disease, including commercial implications of such damage;
(*v*) where the crop (resource) is grown;
(*vi*) area planted to crop (resource);
(*vii*) public health, environmental, aesthetic or nuisance implications of the pest or disease activity; and
(*viii*) economic considerations, including description of the market sector, value of the crop (resource), and potential economic impact of a positive or negative regulatory decision. In cases where more detailed economic analysis is required, proponents should refer to Regulatory Directive DIR93-17, *Assessment of the Economic Benefits of Pesticides*, for guidance.

Part 10.4.3 Current Crop Protection Tools and Practices

The nature of current tools for management of the particular pest or disease problem should be outlined here, including regulatory status, availability in Canada and performance or other benefits or disadvantages relative to the EP proposed for registration.

This information should be presented in the context of unique performance or safety features of the EP that may complement benefits or mitigate drawbacks of current tools, e.g., resistance mitigation, enhancement of environmental or human safety, synergistic activity with conventional tools. Where relevant, reference should also be made to current or proposed crop (resource) management practices that influence the value of the new EP, e.g., crop rotation, zero till, soilless cultivation.

Part 10.4.4 Contribution to Integrated Pest Management Strategies and Practices

EPs may exhibit features, e.g., relatively narrow host range or limited environmental persistence, that constitute a key element in the development and implementation of IPM strategies. Based on the EP profile, host range and reported performance data, the actual or potential role of the new EP in integrated management should be described. The discussion should consider the current status of integrated management practices in the specific crop or resource production system and how the new EP may contribute to the development or implementation of more integrated or sustainable practices. Constraints to implementation of integrated tactics should also be discussed where relevant.

As an essential element of IPM today, the role of the new EP in resistance management should be addressed, both from the perspective of mitigation of resistance to conventional or current products as well as potential for development of pest resistance to the MPCA. Information on the possible development of resistance should be provided. Where development of pest resistance is a known factor, e.g., *Bacillus thuringiensis* (Bt) products, use recommendations should include advice regarding measures to mitigate resistance development.

In the case of EPs in which the deployment of resistant plants expressing protein active ingredients closely related to the MPCA are a factor in management of the proposed host pests or crops, e.g., Bt plants, strategies for integrated use of the EP and the resistant plants should be outlined.

Part 11 Not applicable to microbial pest control products.

Part 12 Summaries

Part 12.5 Foreign Reviews

Foreign reviews should be coded as Part 12.5.x (DACO Part Number) and included at the end of the applicable Part. For example, EPA Reviews of Toxicology would be coded 12.5.4 and included at the end of the information provided for Part 4, *Human Health and Safety Testing*.

Part 12.7 Comprehensive Data Summaries (OECD Dossier Tiers II and III)

Applications for registration for a major new use or new technical grade active ingredient must include comprehensive data summaries in the near future. Applicants should contact the PMRA for the applicability of this requirement when they are ready to submit data for review. For more detailed information on comprehensive data summaries, refer to Regulatory Directives DIR96-05 and DIR97-01, *Comprehensive Data Summaries*. The general format of the summaries should follow those described in the European Commission (EC) guidelines, i.e., based on the EC Directive Tier II, Annex II format as well as Tier III, Annexes II and III. *It is strongly recommended that the applicant consult with the PMRA before preparing the data summaries.* The main objective of the summaries is to provide reviewers with a clear comprehensive summary of characteristics, risks and values associated with the proposed product. The summary should be in such detail that it is apparent that the submitter has performed a thorough evaluation and interpretation of the data of each study. Based on this information, conclusions regarding the safety and potential concerns of the product or active ingredient should be reported.

Comprehensive data summaries should be submitted in a separate binder(s) clearly labelled as containing comprehensive data summaries. The label should conform to the requirements listed in Section 5, Part 4.2, *Infectivity and Toxicity* of these guidelines.

List of Abbreviations

CR	conditionally required
DACO	data code
DNA	deoxyribonucleic acid
EC	European Commission
EC_{50}	concentration that effects 50% of the population
ED_{50}	dose that effects 50% of the population
Eh	redox potential
ELISA	enzyme linked immunosorbant assay
EP	end-use product
EU	European Union
FDA	*Food and Drugs Act*
FI	formulation intermediate
GEM	genetically engineered microorganism
GLP	Good Laboratory Practice
HC	Health Canada
HPLC	high pressure liquid chromatography
ICMFS	International Commission on Microbiological Specifications for Foods
IP	intraperitoneal
IPM	integrated pest management
IRM	integrated resistance management
IV	intravenous
LCC	lower challenge concentration
LC_{50}	concentration that kills 50% of the population
LC_{95}	concentration that kills 95% of the population
LD_{50}	dose that kills 50% of the population
LT_{50}	time for 50% of the population to die
MAR	maximum application rate
MCC	maximum challenge concentration
MP	manufacturing-use product
MPCA	microbial pest control agent
MRL	maximum residue limit
MSDS	Material Safety Data Sheet
OECD	Organisation for Economic Co-operation and Development
PCPA	*Pest Control Products Act*
PMRA	Pest Management Regulatory Agency
QA	quality assurance
QC	quality control
R	required
SHE/SAV7	primary Syrian hamster embryo system
TGAI	technical grade of active ingredient
U.S. EPA	United States Environmental Protection Agency

Appendix I Data Code (DACO) for Microbial Pest Control Products

Data Code (Part)	Title	Data Required	U.S. EPA Guidelines Reference Number	Additional Information or Conditions
M0	**Index**			
M1	**Label, Product Profile, Proposed Use Patterns and International Regulatory Status**			
M1.1	Label		40CFR 152.50 40CFR 156	
M1.2	Product profile and proposed use pattern		40CFR 158.740a 40CFR 152.50 40CFR 156	
M1.3	International regulatory status of the MPCA and EP			
M2	**Product Characterization and Analysis**			
M2.1	Name and address of applicant		40CFR 152.50	
M2.2	Name and address of manufacturing plant			
M2.3	Name and address of formulating plant (if different from 2.2)			
M2.4	Trade name		885.1100	
M2.5	Binomial name (MPCA)			
M2.6	Canadian patent status information			
M2.7	Characterization of the MPCA			
M2.7.1	Origin, derivation, and identification of MPCA(s)		885.1100	
M2.7.2	Biological properties of MPCA(s)		885.1100	
M2.7.3	Characterization of MPCAs derived through recombinant nucleic acid technologies			
M2.7.3.1	Taxonomy and characterization of host and donor microorganisms			
M2.7.3.2	Construction of the recombinant microorganism			
M2.7.3.3	Nature and expression of introduced or modified genetic material			

Data Code (Part)	Title	Data Required	U.S. EPA Guidelines Reference Number	Additional Information or Conditions
M2.7.3.4	Phenotypic characterization of the modified microorganism			
M2.8	Manufacturing methods and quality assurance		885.1100 885.1200	
M2.9	Disclosure of ingredients			
M2.9.1	Product specifications		885.1500	
M2.9.2	Potency estimation and product guarantee		885.1500	
M2.9.3	Unintentional ingredients		885.1500	
M2.10	Analytical data and methodology			
M2.10.1	Active ingredient or MPCA		885.1300	
M2.10.2	Analysis for microbial contaminants		885.1300	
M2.10.3	Analysis for other unintentional ingredients		885.1300	
M2.11	Storage stability testing		885.2400	
M2.12	Summary of physical and chemical properties		885.1600	
M2.13	Characterization and Analysis Requirements for new EPs of registered MPCAs			
M2.14	Other studies and data			
M4	**Human Health and Safety Testing**			
M4.1	Summary			
M4.2	Infectivity and toxicity			
M4.2.1	Summary		885.3050	
M4.2.2	Acute oral infect. and toxicity		885.3050	
M4.2.3	Acute pulmonary infect. and toxicity		885.3150	
M4.3	Acute infectivity (IV or IP)			
M4.3.1	Summary			
M4.3.2	IV infectivity (e.g., bacterial or viral)		885.3200	
M4.3.3	Intraperitoneal infectivity (e.g., fungae or protozoa)		885.3200	

Data Code (Part)	Title	Data Required	U.S. EPA Guidelines Reference Number	Additional Information or Conditions
M4.4	Acute dermal toxicity		885.3100	
M4.5	Irritation			
M4.5.1	Summary			
M4.5.2	Dermal Irritation study		870.2500	
M4.6	Reporting of hypersensitivity incidence		885.3400	
M4.7	Tissue culture (viral agents only)			
M4.8	Genotoxic potential			
M4.9	Other studies and data			
M5.0	**Exposure Assessment**			
M7.0	**Food and Feed Residue Studies**			
M8	**Environmental Fate**			
M8.1	Summary			
M8.2	Laboratory studies			
M8.2.1	Pure culture testing			
M8.2.2	Microcosm testing			
M8.3	Greenhouse studies			
M8.4	Field studies		885.5200	
M8.5	Other studies and data		885.5300	
M9	Environmental Toxicology			
Each study should be denoted by the appropriate DACO number followed by a Roman numeral suffix (i.e., I, II, III, IV) to indicate the applicable tier level of the data.				
M9.1	Summary			
M9.2	Birds			Specifics of required test depend on level of testing **Tier I**: use maximum challenge concentration **Tier II**: use a lower concentration required on a case by case basis depending on results in Tier 1 **Tier III**: definitive toxicity testing or life cycle testing **Tier IV**: Small scale field studies
M9.2.1	Avian oral toxicity		885.4050	See 9.2, above.

Data Code (Part)	Title	Data Required	U.S. EPA Guidelines Reference Number	Additional Information or Conditions
M9.2.2	Avian pulmonary, inhalation or injection		885.4100	See 9.2, above.
M9.3	Wild mammals		885.4150	See 9.2, above.
M9.4	Fish			
M9.4.1	Freshwater fish		885.4200	See 9.2, above.
M9.4.2	Estuarine or marine fish		885.4280	See 9.2, above.
M9.5	Arthropods			
M9.5.1	Terrestrial arthropods		885.4340	See 9.2, above.
M9.5.2	Aquatic arthropods		885.4240	See 9.2, above.
M9.6	Non-arthropod invertebrates			See 9.2, above.
M9.7	Microorganisms			See 9.2, above.
M9.8	Plants			
M9.8.1	Terrestrial plants		885.4300	See 9.2, above.
M9.8.2	Aquatic plants		885.4300	See 9.2, above.
M9.9	Other studies and data			
M10	**Value (Including Efficacy)**			
M10.1	Summary			
M10.2	Performance assessment			
M10.2.1	Lab or growth chamber studies			
M10.2.2	Field studies		810.1000	
M10.3	Treatment effects			
M10.3.1	Phytotoxicity and phytopathogenicity			
M10.3.2	Compatibility with crop protection and management practices			
M10.3.2.1	Effects on MPCA performance			
M10.3.2.2	Effects of the EP			
M10.4	Crop or Resource Production Benefits			
M10.4.1	Profile of the EP			
M10.4.2	Nature and economics of pest or disease problem in Canada			
M10.4.3	Current crop protection tools and practices			

Data Code (Part)	Title	Data Required	U.S. EPA Guidelines Reference Number	Additional Information or Conditions
M10.4.4	Contribution to IPM strategies and practices			
M10.5	Other studies and data			
M12 Summaries				
M12.5	Foreign reviews			Please code 12.5. (Canadian Daco Part No.) and include at end of applicable part
M12.7	Comprehensive summaries		885.0001	

Appendix II Elements of a Complete Submission to Register, to Amend or to Conduct Research With a Pest Control Product

Some of the elements listed below are conditionally required depending on the purpose of the submission. For more information, refer to the *Registration Handbook for Pest Control Products Under the Pest Control Products Act and Regulations*.

Required Elements include:

(a) **Covering letter:** outlining the purpose of the submission and a brief description of the submitted package. It should include the product name, a brief description of the intended use of the product, a reference to related submissions, and relevant history, if applicable. Data should not be included as part of the covering letter. A distinct letter should be included with each submission.

The covering letter must be submitted with the envelope (refer to Section 4.2). A copy of the covering letter must be included in the Summary Binder. Copies of the covering letter should not be included in other data binders.

(b) **Application form:** completed, signed and dated.

(c) **Fee:** as indicated on the application form in a cheque payable to the Receiver General for Canada.

Conditionally Required Elements include:

(a) **Product specification form:** completed, signed and dated.

(b) **Letter(s) of confirmation:** of source of active ingredient(s).

(c) **Letter(s) of authorization:** to cite data previously submitted by another company.

(d) **Letter(s) of authorization:** designating agent, formulator, consultant, etc.

(e) **Letter(s) of authorization:** to share data reviews with other countries.

(f) **Draft label:** in the proper electronic and paper formats. See Section 5, Part 1.1 and Appendix IV.

(g) **Index:** of supporting data in the proper electronic and paper format. See Appendix III.

(h) **Scientific data or studies:** supporting the safety and effectiveness of the proposed product or amendment.

(i) **Foreign reviews:** of the submitted scientific data or studies, if available.

(j) **Comprehensive data summary:** in accordance with the EC guidelines. Refer to Regulatory Directives DIR96-05 and DIR97-01, *Comprehensive Data Summaries*.

(k) **Requests for waivers:** from the requirement of producing and submitting specific scientific data/studies. Such requests must be recorded in the index and supported by surrogate data or a scientific rationale in place of the DACO or study.

Appendix III Directions for Creating a Data Index

Electronic Format

All data and supporting information submitted, including requests for waivers, surrogate studies, foreign reviews, protocols, study screens, and literature, should be listed in the index in the format outlined below. Field titles should not be included in the index. Refer to the examples that follow.

Field		Information
DACO Number	-	corresponding Canadian DACO number (Please refer to Appendix I)
Author(s)	-	surname, initial(s)
Date (Year)	-	year report written by laboratory (not year submitted)
Title	-	full title as it appears in the report
Testing Lab Name	-	where different from company name
Testing Lab Report No.	-	Lab Report Number assigned by the testing laboratory
Full Date	-	give date of report as day, month, year, where date is written out in full
Company Name	-	of data submitter/data owner
Company Report No.	-	Company Report Number assigned by the Company
Volume No. of Data Part	-	volume number for the particular data part
No. of Pages	-	total number of pages of the study
City	-	of data submitter/data owner
Country	-	of data submitter/data owner
Published/Unpublished	-	state whether the material is published or unpublished
Date of Submission	-	day, month, year
EPA MRID No.	-	if available
Comments	-	Comments of the Company, e.g., foreign review, cross-referencing

Applicants should submit a 3.5" diskette, with the index information saved in a valid WordPerfect or ASCII delimited text format. When applicants wish to author the index in a different electronic environment, it is the applicant's responsibility to confirm that no text loss or format changes have occurred as a result of the conversion. Format should be a hard return for each line with a double hard return between each study. Repeated as many times as there are studies.

The diskette should be labelled with the following information:

- name of the registrant
- product name
- scientific name and strain designation of the MPCA
- part number and title
- date of submission
- diskette format, i.e., WordPerfect or ASCII

Default Entries

Where standard citation information is not available or is unclear, a default entry of N/S acceptable. Exceptions include:

- Where authors are not identified, use Anonymous.
- When the report number is known but not the authors, it may be possible to reference the name of the study director.

Viruses

Note: Applicants are required to provide diskettes that are certified virus-free. Any diskette found to contain a virus will be returned to the applicant.

Examples of Index Entries

4.3.5
Hartley, M. and Murray, W.
(1994)
S-1234 (Technical Grade) twenty-one day dermal study in rabbit
Happy Labs., United Kingdom
Report Number 007
Report Date 25 January 1994
Pesticide Company 1
N/S
N/S
58 Pages
Bilthoven
Netherlands
Unpublished
26 March, 1994
N/S
N/S

4.6.4
Anonymous
(1985)
Eye Sensitivity Studies with 20% EC
Huntington Research Centre
Report No. FMT6-85539
Report Date January 16 1985
Pesticide Company 2
Company Report No. 5
Volume 23
88 Pages
N/S
England

Unpublished
April 12, 1994
N/S
N/S

Hard Copy (paper) Format

An abbreviated index may be submitted as the hard copy version provided it contains the following information:

DACO
Author
Date (year)
Title (underlined)
Location of study (i.e., part number, volume number, tab number in the volume)
Total number of pages of the study or DACO
Submission Date
Comments

Example:

4.3.5 Hartley, M. and Murray, W. (1994) S-1234 (Technical Grade) twenty one day dermal study in rabbit, Part 4, Vol. 1, tab 4.3.5. (58 pages). March 21, 1997.

Appendix IV Directions for Creating a Draft Label

Basic label requirements are outlined below. For more detailed information on the preparation of product labels, refer to the *Registration Handbook for Pest Control Products Under the Pest Control Products Act and Regulations* (Registration Handbook).

Principal Display Panel

1. Product Name

- Must match name on the application form
- Should be specific to the product and be descriptive of its physical form and purpose
- Should not be misleading or contain unacceptable or scientifically unsupportable adjectives, e.g., Natural, Organic, etc.

2. Class Designation

- Based on intended use and potential hazards
- Must match class indicated on application form
- Usually only one class designation accepted per product (there are some exceptions with combined commercial and manufacturing, commercial and restricted, etc.)
- Class designation must be one of the following:
  - DOMESTIC
  - COMMERCIAL (AGRICULTURAL, INDUSTRIAL or INSTITUTIONAL)
  - RESTRICTED
  - MANUFACTURING

3. Precautionary Symbols and Words (refer to criteria in the Registration Handbook)

- This information is not usually necessary for microbial products; however, if required according to the criteria presented in the Registration Handbook, *Poison*, *Flammability*, *Explosive*, and *Corrosive* hazard symbols and signal words must appear on the label.

4. *READ THE LABEL BEFORE USING* Statement

- If the product labelling includes a brochure or pamphlet, this should be indicated on the label, i.e., *READ THE LABEL AND BROCHURE BEFORE USING.*

5. *GUARANTEE* Statement

- The guarantee on the label must match that on the product specification form and both of these are to reflect the concentration of the active ingredient(s) as described in Part 2.9.2 of these guidelines.

6. Registration Number

- The registration number on the label must match the one assigned.
- This number must appear as: *REGISTRATION NUMBER XXXXX PEST CONTROL PRODUCTS ACT*, or if it is a domestic class product and size is a limiting factor, as *REG. NO. XXXXX P.C.P. ACT*.

7. *Net Contents*

- Must be expressed in metric units (imperial measure may appear in brackets after the metric measure)
- Liquids are expressed in millilitres (mL) or litres (L) and solids or pressurized products are expressed in grams (g) or kilograms (kg).

8. *Name and Full Postal Address of Registrant*

- Must match that in box 6 of the application form
- The name and address of the Canadian agent is required on the label if the registrant resides outside of Canada.
- The registrant's telephone number is required on the label of technical or manufacturing products.

Note: For domestic class products of very small size, points 5, 6, 7 and 8, listed above, can appear on the secondary display panel.

Secondary Display Panel

1. *DIRECTIONS FOR USE*

- Must include complete information on application rates, how to apply the product, and use limitations.
- For technical or manufacturing products, the standard *DIRECTIONS FOR USE* statement is: *To be used only in the manufacture of a pesticide which is registered under the Pest Control Products Act. Read Technical Bulletin for formulation details*. (Please note that the word *pesticide* can be replaced with biological insecticide, herbicide, fungicide, etc.)

2. *PRECAUTIONS*

- Must include information on any significant hazard relating to handling, storage, display, or distribution of the product, and how to alleviate such hazards
- Must include any significant hazard relating to human health, wildlife, or the environment that may result from the use of the product, along with instructions on how to alleviate such hazards
- Should include the statement: KEEP OUT OF REACH OF CHILDREN

3 and 4. *FIRST AID* and *TOXICOLOGICAL INFORMATION*

- This information is not normally necessary for labels of microbial products, but a clear and concise statement of practical first aid measures will be required in cases where the product could pose a hazard as the result of accidental contact with skin or eyes, or ingestion or inhalation.

5. *STORAGE STATEMENT*

- Must include information on appropriate storage conditions (e.g., temperature range and light restrictions) and any other relevant information aimed at ensuring product stability, performance and safety.

6. *DISPOSAL*

(a) For products of DOMESTIC class designation:

Do not reuse empty container. Dispose of empty container with household garbage.

(b) For products of COMMERCIAL class designation:

Liquid:

1. *Rinse the emptied container thoroughly and add the rinsing to the spray mixture in the tank.*
2. *Follow provincial instructions for any required additional cleaning of the container prior to its disposal.*
3. *Make the empty container unsuitable for further use.*
4. *Dispose of the container in accordance with provincial requirements.*
5. *For information on the disposal of unused, unwanted product and the cleanup of spills, contact the provincial regulatory agency or the manufacturer.*

Solid:

1. *Thoroughly empty the contents of the container into the application device.*
2. *Make the empty container unsuitable for further use.*
3. *Dispose of the container in accordance with provincial requirements.*
4. *For information on the disposal of unused, unwanted product and the cleanup of spills, contact the provincial regulatory agency or the manufacturer.*

(c) For products of technical or manufacturing class designation:

Canadian formulators of this technical should dispose of unwanted active and containers in accordance with municipal or provincial regulations. For additional details and the cleanup of spills, contact the provincial regulatory agency or the manufacturer.

7. *NOTICE TO USER*

- The Notice to User statement, below, is required on all COMMERCIAL, RESTRICTED and MANUFACTURING class products:

  This control product is to be used only in accordance with the directions on this label. It is an offense under the Pest Control Products Act to use a control product under unsafe conditions.

8. *NOTICE TO BUYER* (optional)

- If a registrant wishes to include a notice to buyer statement on the label, the wording must be as follows:

  Seller's guarantee shall be limited to the terms set out on the label and, subject thereto, the buyer assumes the risk to persons or property arising from the use or handling of this product and accepts the product on that condition.

SAMPLE DRAFT LABEL

PRINCIPAL PANEL

LEP BE-GONE
Flowable Biological Insecticide

RESTRICTED For use in Forestry

READ THE LABEL BEFORE USING
KEEP OUT OF REACH OF CHILDREN

GUARANTEE: *Bacillus thuringiensis* var. *kurstaki*, strain RL 99: 10,000 International Units of potency per mg (equivalent to 12 billion International Units of potency per litre)

REGISTRATION NO: XXXXX PEST CONTROL PRODUCTS ACT

Precautionary symbols and signal words (if appropriate)

Net Contents: 1 L

XYZ Biologicals Inc.
Postal Address
City Province Postal Code

Lot Number (if required)
Expiry Date (if required)

SECONDARY PANEL

NOTICE TO USER: This control product is to be used only in accordance with the directions on this label. It is an offense under the *Pest Control Products Act* to use a control product under unsafe conditions.

NATURE OF RESTRICTION: This product is to be used only in the manner authorized. Consult local pesticide regulatory authorities about use permits which may be required.

RESTRICTED USE: For use against spruce budworm larvae in forests.

DIRECTIONS: Treat when larvae are feeding. Do not mix with any other materials. Spray foliage at rate of 1 L/ha. Provide a uniform deposit on foliage; larvae must eat deposit of LEP BE-GONE to be affected. Recommended droplet size is 100 µm.

PRECAUTIONS: KEEP OUT OF REACH OF CHILDREN. Avoid contact with skin, eyes and clothing. Wash with soap and water after use.

FIRST AID: In case of contact, flush skin or eyes with clean water. If irritation persists, obtain medical attention or contact a poison control centre.

STORAGE: Store at temperatures between 0°C and 15°C. Store container upright and keep tightly closed when not in use. After extended storage, vigorously shake or stir contents to assure a uniform suspension.

DISPOSAL: Do not reuse container. Follow provincial instructions for any required cleaning of the container prior to its disposal. Make empty container unsuitable for use and dispose in accordance with provincial requirements. For information on the disposal of unused, unwanted product and the cleanup of spills, contact the provincial regulatory agency or the manufacturer.

Appendix V Required numbers of copies of information supporting submissions for microbial pest control products

Part No.	Data and Information	Reviewing Division				Total No. of Copies
		SMID	HED	EAD	PSCD	
0	Index[1,3]	1	1	1	1	4
1	Label[1,2,3] Product profile and use pattern[1,3] Summaries[3] MSDSs[3]	1	1	1	1	4
2	Product Characterization and Analysis	1			1	2
4	Human Health and Safety Testing	1	1			2
5	Exposure Assessment	1	1			2
7	Food and Feed Residue Studies	1	1			2
8	Environmental Fate	1		1		2
9	Environmental Toxicology	1		1		2
10	Value	1			1	2
12	Comprehensive Data Summaries	1	1	1	1	4

[1] One electronic copy as well as hard (paper) copies in the format prescribed in this document are required.

[2] Two additional paper copies of the label are required and should be submitted in the envelope with the cover letter, i.e., not in binders but with the non-data submission components.

[3] These items are to be combined in the summary binder.

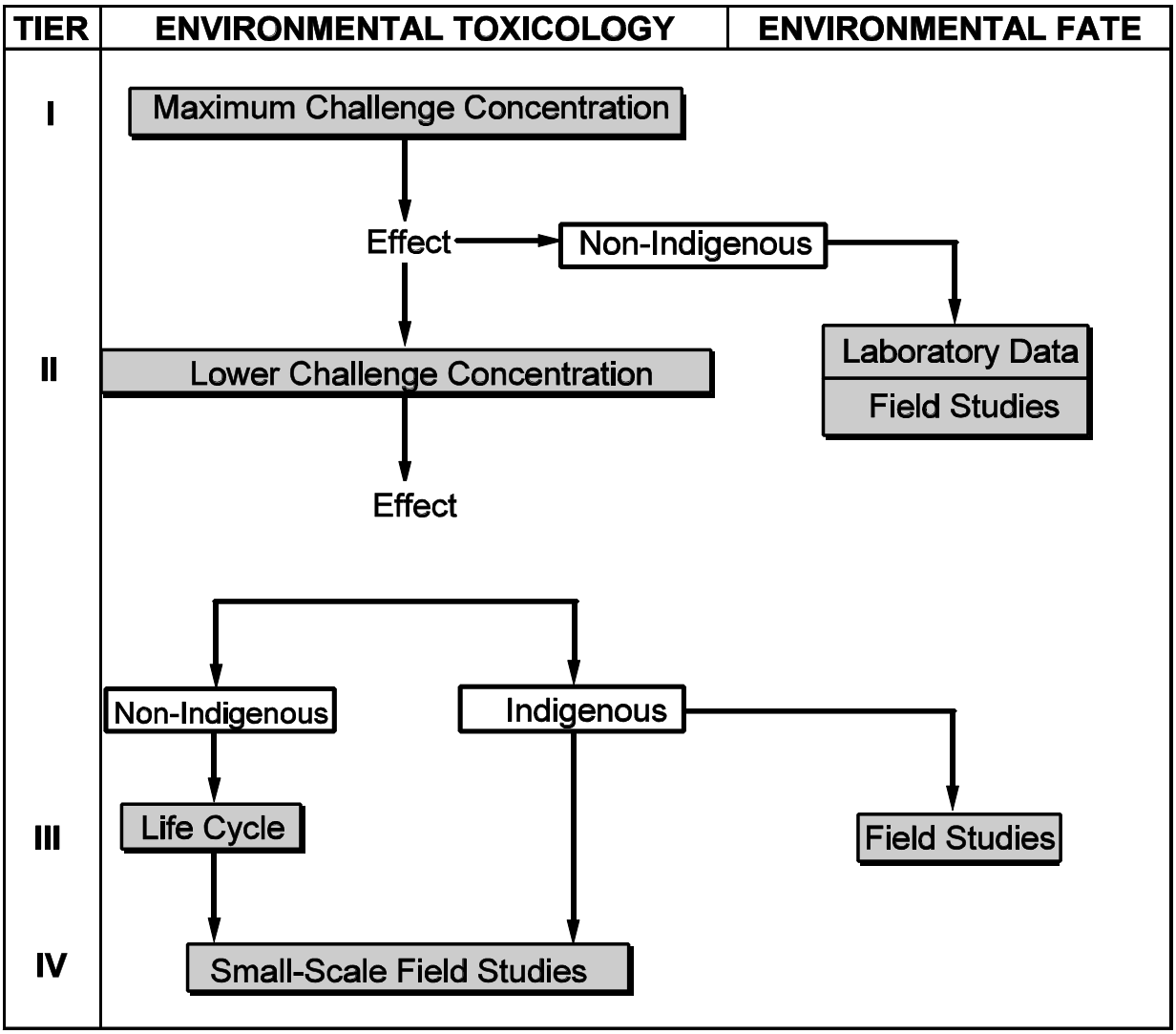
Acronyms
EAD: Environmental Assessment Division
HED: Health Evaluation Division
PSCD: Product Sustainability and Coordination Division
SMID: Submission Management and Information Division

Appendix VI Human Health and Safety Testing Requirements

Test Animal Species	DACO	Data Required	Test Substance
Part 4 Human Health and Safety Testing			
Summary	4.1	R	
Infectivity and toxicity	4.2		
Summary	4.2.1	R	
Acute oral: Rat (preferred)	4.2.2	R	TGAI
Acute pulmonary: Rat (preferred)	4.2.3	R	TGAI
Acute IV or IP infectivity	4.3		
Summary	4.3.1	R	
Intravenous infectivity (bacteria or viruses): Newly weaned mouse or hamster	4.3.2	R	MPCA
Intraperitoneal infectivity (fungi or protozoa): Rat or mouse	4.3.3	R	MPCA
Acute dermal toxicity: Rabbit	4.4	R	EP
Irritation	4.5		
Summary	4.5.1	R	
Dermal irritation study: Rabbit	4.5.2	R	EP
Reporting of hypersensitivity incidence	4.6	R	MPCA or EP
Tissue culture (viral agents only)	4.7	R	MPCA
Genotoxic potential (fungi or actinomycetes)	4.8		MPCA

Appendix VII Microbial Pesticide Ecozones of Canada

Appendix VIII Environmental Toxicology and Environmental Fate Testing Tiers



Appendix IX Environmental Toxicology and Fate Testing Requirements

Test	DACO	Use Pattern				Test Substance	Type of Test
		Terrestrial	Aquatic	Forestry, Domestic Outdoor	Greenhouse*		
Tier I							
Avian Oral	9.2.1	R	R	R	CR	TGAI or EP	MCC
Avian Pulmonary, Inhalation or Injection	9.2.2	R	R	R	CR	TGAI or EP	MCC
Wild Mammals	9.3	CR	CR	CR	CR	TGAI or EP	MCC
Fish: Freshwater	9.4.1	R	R	R	CR	TGAI or EP	MCC
Fish: Estuarine or Marine	9.4.2	CR	CR	CR	CR	TGAI or EP	MCC
Arthropods: Terrestrial	9.5.1	R	R	R	CR	TGAI or EP	MCC
Arthropods: Aquatic	9.5.2	R	R	R	CR	TGAI or EP	MCC
Non-Arthropod Invertebrates: Terrestrial	9.6.1	R	R	R	CR	TGAI or EP	MCC
Non-Arthropod Invertebrates: Aquatic	9.6.2	R	R	R	CR	TGAI or EP	MCC
Microorganisms	9.7	CR	CR	CR	CR	TGAI or EP	MCC
Plants: Terrestrial	9.8.1	R	R	R	CR	TGAI or EP	MCC
Plants: Aquatic	9.8.2	R	R	R	CR	TGAI or EP	MCC
Tier II							
Avian Oral	9.2.1	CR	CR	CR	CR	TGAI or EP	LCC
Avian Pulmonary, Inhalation or Injection	9.2.2	CR	CR	CR	CR	TGAI or EP	LCC
Wild Mammals	9.3	CR	CR	CR	CR	TGAI or EP	LCC
Fish: Freshwater	9.4.1	CR	CR	CR	CR	TGAI or EP	LCC
Fish: Estuarine or Marine	9.4.2	CR	CR	CR	CR	TGAI or EP	LCC

Test	DACO	Use Pattern				Test Substance	Type of Test
		Terrestrial	Aquatic	Forestry, Domestic Outdoor	Greenhouse*		
Arthropods: Terrestrial	9.5.1	CR	CR	CR	CR	TGAI or EP	LCC
Arthropods: Aquatic	9.5.2	CR	CR	CR	CR	TGAI or EP	LCC
Non-Arthropod Invertebrates: Terrestrial	9.6.1	CR	CR	CR	CR	TGAI or EP	LCC
Non-Arthropod Invertebrates: Aquatic	9.6.2	CR	CR	CR	CR	TGAI or EP	LCC
Microorganisms	9.7	CR	CR	CR	CR	TGAI or EP	LCC
Plants: Terrestrial	9.8.1	CR	CR	CR	CR	TGAI or EP	LCC
Plants: Aquatic	9.8.2	CR	CR	CR	CR	TGAI or EP	LCC
Environmental Fate: Pure Culture Testing	8.2.1	CR	CR	CR	CR	MPCA	NA
Environmental Fate: Microcosm Testing	8.2.2	CR	CR	CR	CR	TGAI or EP	MCC
Environmental Fate: Small- or Large-Scale Field Studies	8.2.4	CR	CR	CR	CR	EP	MAR
Tier III							
Definitive Toxicity Testing of Non-Target Organisms	9.2.1–9.8.2	CR	CR	CR	CR	TGAI or EP	Multiple Concentra-tions
Life Cycle Testing of Non-Target Organisms	9.2.1–9.8.2	CR	CR	CR	CR	TGAI or EP	EEC
Environmental Fate: Small- or Large-Scale Field Studies	8.2.4	CR	CR	CR	CR	EP	MAR
Tier IV							
Environmental Toxicology: Small-Scale Field Studies	9.2.1–9.8.2	CR	CR	CR	CR	EP	MAR

* For greenhouse uses, the need for environmental testing will depend largely on the type (i.e., the design and operation of greenhouse facilities in which the EP will be applied) and the degree of environmental exposure (i.e., the level of containment) anticipated under operational conditions of use. Advance consultation with the PMRA is recommended to identify specific data requirements for the MPCA in question.

Appendix X Non-target Toxicology Testing

Tier	Type of Test	Form of Microbial Agent	Non-target to be Tested
I	Maximum Challenge Concentration	TGAI or EP	1. Taxonomically related 2. Infected by MPCA 3. High exposure potential 4. Similar physiology 5. Susceptible to related pathogens 6. Representative species from 7 broad taxonomic groups
II	Lower Challenge Concentration	TGAI or EP	Adversely affected species from Tier I toxicology tests
III*	Determination of an LC_{50}, LD_{50}, EC_{50} and Life Cycle Tests	TGAI or EP	Adversely affected species from Tier II toxicology tests
IV	Small-Scale Field Studies (Environmental toxicology)	EP	Adversely affected species from Tier II toxicology tests

* Tier III testing is not required for indigenous MPCAs

Appendix XI Suggested Taxa for Selection of Non-target Arthropods

Group	Freshwater	Estuarine or Marine	Terrestrial
Arachnida	Araneae		Araneae Scorpionida
Acari			Eriophyidae Phytoseiidae Stigmaeidae Tetranychidae Tydeoidae
Crustacea	Cladocera Copepoda Decapoda Amphipoda	Anostraca Copepoda Cirripedia Mysidacea Amphipoda Decapoda	Isopoda
Insecta	Ephemeroptera Odonata Plecoptera Megaloptera Trichoptera Lepidoptera Coleoptera Diptera Hymenpotera		Collembola Thysanura Dictyoptera Isoptera Grylloptera Orthoptera Psocoptera Hemiptera Heteroptera Homoptera Thysanoptera Neuroptera Coleoptera Diptera Hymenoptera Lepidoptera

Appendix XII Suggested Taxa for Selection of Non-target Plant Species

Terrestrial	Aquatic
Apiaceae (Umbelliferae)	Lemnaceae
Asteraceae (Compositae)	Potomogetonaceae
Brassicaceae (Cruciferae)	Haloragaceae
Chenopodiaceae	Typhaceae
Cucurbitaceae	Cyperaceae
Fabaceae (Leguminosae)	Alismaceae
Liliaceae	
Malvaceae	
Poaceae (Gramineae)	
Polygonaceae	
Rosaceae	
Solanaceae	

Appendix XIII List of Relevant Publications

Regulatory Authority
Pest Control Products Act and Regulations
Food and Drugs Act and Regulations

PMRA Companion Guidance Documents
Registration Handbook for Pest Control Products Under the Pest Control Products Act and Regulations (Registration Handbook)

Regulatory Proposal PRO93-05, *Research Permit Guidelines for Microbial Pest Control Products*

Regulatory Directive DIR93-07*a*, *Guidelines for Efficacy Assessment of Chemical Pesticides*

Regulatory Directive DIR93-07*b*, *Guidelines for Efficacy Assessment of Herbicides and Plant Growth Regulators*

Regulatory Directive DIR93-17, *Assessment of the Economic Benefits of Pesticides*

Regulatory Directive DIR96-01, *Guidelines for Efficacy Assessment of Fungicides, Bactericides and Nematacides*

Regulatory Proposal PRO96-01, *Management of Submissions Policy*

Regulatory Directive DIR98-01, *Good Laboratory Practice*

Regulatory Directives DIR96-05 and DIR97-01, *Comprehensive Data Summaries*

Instructions for Organizing and Formatting a Complete Submission Package for Pest Control Products (pending publication)

Other publications of interest
International Commission on Microbiological Specifications for Foods (ICMSF). 1978. *Microorganisms in Foods, 1. Their Significance and Methods of Enumeration*. 2nd Edition. International Commission on Microbiological Specifications for Foods. University of Toronto Press, Toronto (ISBN 0-8020-2293-6).

International Commission on Microbiological Specifications for Foods (ICMSF). 1986. *Microorganisms in Foods, 2. Sampling for Microbiological Analysis. Principles and Specific Applications*, 2nd Edition. International Commission on Microbiological Specifications for Foods. University of Toronto Press, Toronto (ISBN 0-8020-5693-8).

Laboratory Biosafety Guidelines (2nd Edition, 1996, Health Canada, Ottawa, Ontario, ISBN: 0-662-24214-9)

OECD Guidelines for Testing of Chemicals # A-404, Acute Dermal Irritation, 1993