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Guidance for the Registration of Microbial Pest Control Agents and Products

This guidance document outlines the requirements for the registration of microbial pest control agents and products proposed for pest management in Canada

PMRA Guidance Document



*Protecting the health and
environment of Canadians*

*Protéger la santé des Canadiens
et l'environnement*



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17 November 2021	Revised to update content and wording to align with current requirements for registration of microbial pest control agents and their products. This document replaces DIR2001-02, <i>Guidelines for the Registration of Microbial Pest Control Agents and Products</i> .
March 2001	Publication of original document: DIR2001-02, <i>Guidelines for the Registration of Microbial Pest Control Agents and Products</i> .

Disclaimer

This document does not constitute part of the *Pest Control Products Act* or its regulations and in the event of any inconsistency or conflict between the Act or regulations and this document, the Act or the regulations take precedence. This document is an administrative document that is intended to facilitate compliance by the regulated party with the Act, the regulations and the applicable administrative policies.

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1.0 Purpose

This guidance document outlines the requirements for the registration of microbial pest control agents (MPCAs) and end-use products proposed for pest management in Canada at this time. MPCAs are naturally occurring or genetically modified microorganisms, including bacteria, algae, fungi, protozoa, viruses, mycoplasmae or rickettsiae, and related organisms.

2.0 Scope

This guidance document is meant to assist the registration of MPCAs that have the potential to contribute to alternative pest management by identifying data requirements that are specifically developed for these types of active ingredients.

This guidance document supports 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.

3.0 Introduction

The purpose of this document is to provide guidance on the scientific and technical information required to register MPCAs and associated pest control products. The information presented for MPCAs and end-use products should be considered as supporting information to the *Pest Control Products Act* and *Pest Control Products Regulations*. Applicants should carefully review this document and consult with the PMRA prior to making a registration submission.

The registration requirements outlined in this guidance document were developed to address the assessment of human health risk, environmental risk, and value of end-use products that contain naturally occurring and genetically modified bacteria, algae, fungi, protozoa, viruses, mycoplasmae or rickettsiae, and related organisms, as the active ingredient. Because of the specific nature of these active ingredients, PMRA's assessment of these products includes an evaluation of aspects such as product characterization, manufacturing and quality control, which are distinct from those of conventional pest control products. In other words, the organization of the information parallels that for conventional pest control products while taking into account unique aspects of these types of active ingredients, such as biological properties, host range, potential pathogenicity, infectivity and the abilities to persist, multiply and disseminate in the environment.

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 requests for certain data requirements will be considered when accompanied by a sound scientific rationale.

Health Canada's PMRA and the United States Environmental Protection Agency (USEPA) are committed to joint reviews and work sharing of microbial pesticide evaluations on a regular basis. Consequently, the PMRA and the USEPA 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 increase the efficiency of the registration process, facilitate simultaneous registration in Canada and the United States, and increase access to new pest management tools in both countries. Efficient work sharing 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 [Pesticides section](#) of Canada.ca.

For research conducted with microbial pest control products, researchers should refer to the guidance, *Research Permit Guidelines for MPCPs* during the planning phase to determine the data required to obtain regulatory approval.

4.0 Pre-submission consultations regarding data requirements

This guidance document contains information regarding the data requirements for a variety of MPCAs and end-use products. Since the data required for registration depend on the identity and biological properties of the MPCA, as well as the nature of the product and the intended use pattern, applicants are encouraged to consult the Data Code (DACO) Table in Appendix II during the developmental phase of their product to gain an understanding of the scope of the data required to support their registration. Once a strategy has been prepared, applicants can request a pre-submission consultation to address specific questions that may concern the MPCA in question. Applicants should contact the Pest Management Information Service (pmra.info-arla@hc-sc.gc.ca) for information on appropriate contacts for pre-submission consultation.

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

If a pre-submission consultation is requested, information must be submitted via e-PRS (see Appendix IV), must include a cover letter requesting a pre-submission meeting and a proposed agenda of the specific issues to be discussed, and at a minimum, the information requested in Section 6, Part M1, Label, Product Profile, Proposed Use Pattern(s) and International Regulatory Status, and Section 6, Part M2.7, 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 II of these guidelines contains a DACO Table that lists all data that may be required for the registration of a microbial product. Applicants should build their data package using this DACO Table, noting 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 upon registration.

5.0 Organizing and formatting a complete microbial submission package

Submission verification

Within seven days of receipt, all submissions are verified to ensure that fees, forms, labels and required information have been provided (see [The Management of Submissions Policy Process](#)). Deficiencies will result in a submission being returned to the applicant. An applicant whose submission is verified is provided with an acknowledgement of the submission number. This number should appear on all correspondence dealing with that submission. Once verified, submissions are forwarded for screening.

Submission screens

Upon receipt by the Submission screening section, a submission for a new MPCA or a major new use of a registered end-use product 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. The submission number is no longer valid. An applicant may resubmit the package as a new submission.

Elements of a complete submission package

Only complete submissions will be considered for review by the PMRA. 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 III 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 5.0, Pre-submission consultations regarding data requirements, for more information).

5.1 Organization of supporting data

Supporting data for registration of microbial products are divided into ten general parts, as outlined below. The numbering of these parts is analogous to those of conventional chemical pesticides, with the "M" designation for microbial active ingredients and products. Certain parts are not identified (Parts 3, 6 and 11), as they are not required for MPCAs. Each major part is further divided into sub-parts, as described in this document and in the DACO table, Appendix II.

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

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, for example, acute oral toxicity and infectivity-waiver request.

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). In this case, it is important that the "comments" field of the index indicate all DACOs for which the data pertains. For example, "Waiver request for acute oral toxicity and infectivity; acute inhalation toxicity and infectivity."

Using literature to address data requirements

Legible copies of relevant papers must be submitted to the PMRA by e-PRS. Each report should be submitted under the appropriate DACO, and indexed as per Appendix IV, Directions for Creating a Data Index. The comments field of the index must be completed, referring to the study as “published”.

A discussion for which the supporting documents were submitted must be made in a separate document which contains a comprehensive reference list citing the published documents and must be submitted under the appropriate DACO.

Additional data

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, for example, a standard operating procedure pertaining to Part M4, Human Health and Safety Testing, should be submitted under DACO M4.9, Other Studies/Data.

Foreign reviews should be coded under DACO M12.5 as outlined in Appendix II, Data Code (DACO) for Microbial Pest Control Products and coded according to its related data part, for example, a foreign review of toxicology data, Part M4, would be coded as DACO M12.5.4 (see Section 6, Part M12.5 of this document).

6.0 Data required for the registration of microbial pest control agents and products

The components of a submission should be organized as outlined below. Separate packages should be submitted for each proposed product, in other words, each technical grade active ingredient, manufacturing concentrate or end-use product. 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. The exception to this would be where the manufacturing concentrate or technical grade active ingredient is identical to the end-use product; in this case, a single package of relevant data may be submitted for these related submissions.”

Each product to be registered, in other words, each technical grade active ingredient or manufacturing concentrate and each end-use product, will be associated with its own data package and its own submission. However, end-use products manufactured using an integrated formulation process wherein the manufacturing concentrate or the technical grade active ingredient is identical to the end-use product, will be associated with a single data package, but under separate submissions.

Part M0 Index

The index must be submitted in electronic format via e-PRS. 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, in other words, the technical grade active ingredient, the manufacturing concentrate and each end-use product, unless the product is manufactured using an integrated formulation process. Please refer to Appendix IV for directions on preparing electronic indices.

Documents that are authored by PMRA and are publicly available (for example, PRVDs, PRDs) must also not be submitted as part of the data package.

Part M1 Label, product profile, proposed use pattern(s) and international regulatory status

Part M1.1 Label

Information specific to microbial pest control products is also provided in Appendix V, Directions for Creating a Draft Label for microbial pest control products. Applicants are advised to consult the Registration Directorate, the 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 6, Part M2.9, 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 6, Part M10, Value assessment.

Part M1.2 Product profile and proposed use patterns

A profile of the MPCA or end-use product 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, including production of secondary metabolites related to the pesticidal activity, and potential pathogenicity or genotoxicity of the MPCA, etc.;
- (iii) concentration range, in other words, minimum and maximum levels, of the MPCA in the technical grade active ingredient and the end-use product(s), in appropriate units;
- (iv) product type, for example, bioinsecticide, biofungicide;
- (v) product class, for example, restricted, domestic, commercial;
- (vi) formulation type, for example, 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, for example, forests, greenhouses, residential areas, aquatic systems;
- (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, time to harvest;
- (xii) application methods and equipment: in other words, 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, for example, aquatic, marine, with reference to risk mitigation measures, as appropriate.

Part M1.3 International regulatory status of the MPCA and the end-use product

The international regulatory status of the MPCA proposed for registration and related end-use products should be reported, including:

- (i) countries of registration;
- (ii) dates registered or status of review;
- (iii) indication if regulatory reviews from the USEPA, the European Union (EU) or other OECD countries are available or submitted (these reviews should be coded as Part M12, 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 M2 Product characterization and analysis

a) Introduction

The purpose of Product characterization and analysis is to adequately characterize the MPCA and to substantiate the production of a consistent, safe, stable and biologically active product. As well, the Product and characterization analysis can provide guidance with respect to the data required under the human health, environment, and value. Clear, accurate and detailed information should be provided to allow evaluators to verify the characteristics of the MPCA and the quality of the end-use product 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 M4, Human health and safety testing, Part M5, Exposure assessment, Part M7, Food and feed residue studies, Part M8, Environmental fate and Part M9, 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 M2. 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 under Part M4 and Part M9.

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 M2.

b) Requirements

Part M2.1 Name and address of applicant

Part M2.2 Name and address of manufacturing plant

Part M2.3 Name and address of formulating plant (if different from Part 2.2)

Part M2.4 Trade name

Part M2.5 Binomial name (MPCA)

Part M2.6 Canadian patent status information: number, date issued, and expiry date, if applicable.

Part M2.7 Characterization of the MPCA

The information requirements outlined in Section 6, Part M2.7.1, Origin, derivation and identification of MPCAs, and Section 6, Part M2.7.2, Biological properties of MPCAs, are applicable to all MPCAs. Section 6, Part M2.7.3, 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 end-use product.

Part M2.7.1 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 strain level should be provided, if at all possible. If not available, provide 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 (for example, agricultural soil; in association with the rhizosphere of a plant; from a plant root), clinical, or food isolate; culture collection; description of isolation procedure, including exact geographical origin of the MPCA isolate (for example, city and country must be specified); 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 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, for example, reversion tendency, rate of plasmid loss, of the altered chromosomal or extrachromosomal entity.

Part M2.7.2 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 certain microorganisms, some of this information may already be available in the published scientific literature. In these cases, a detailed literature review may make up a large part of the data requirement. 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. The PMRA 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 (as listed on the product label and in agreement with information provided under Part M10): pathogenicity, kind of antagonism to target hosts, infective or toxic dose, transmissibility, and information on mode of action including the specific compounds which are responsible for the activity on the target plants, 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 MPCA, including the various forms of the MPCA that may occur, as well as 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, for example, temperature (minimum, maximum and optimum growth temperatures), redox potential (Eh), pH, nutritional dependence, and susceptibility to environmental factors, such as sunlight and desiccation; include also an antimicrobial profile for the MPCA to demonstrate susceptibility/tolerance to antimicrobial agents commonly used in human or veterinary medicine;
- (vii) distinguishing characteristics: 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 M4.2, 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 under Part M2.10.3;
- (xii) for fungi and actinomycetes, characterization data must also include 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. Analytical data should be submitted under Part M2.10.3 while the discussion must be included under Part M2.7.2; 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 M2.7.3 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 M2.7.3.1. Taxonomy and characterization of recipient and donor microorganisms

Taxonomic designation of recipient and donor microorganisms must be validated as outlined in Section 6, Part M2.7.1, Origin, derivation and identification, of the MPCA(s). Characterization information, equivalent to that outlined in Section 6, Part M2.7.2, 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 PMRA officials is recommended to determine the extent of the information required.

Part M2.7.3.2. 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.

Part M2.7.3.3. 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 PMRA 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, for example, consensus sequences, promoters, transcription terminators, potential open reading frames, unusual structural properties. 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 M2.7.3.4. 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 M2.8 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, in other words, 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 6, Part M2.7, 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, for example, 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 end-use products 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, in other words, product release standards, that determine whether a batch of technical active ingredient, manufacturing concentrate, or end-use product will be released for commercial use. Details of sampling programs, including procedures, sample size, stage of manufacturing, 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 of the technical grade active ingredient must be submitted under Part M2.10.2. Data on pilot-scale production batches may be acceptable.

QC data on commercial batches of technical grade active ingredient 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 M2.9 Disclosure of ingredients

Part M2.9.1 Product specifications

Every Application for New or Amended Registration of a technical grade active ingredient and an end-use product must each be accompanied by a complete and accurate Statement of Product Specification Form (SPSF)

Under Part M2.9.1, refer to SPSFs that have been submitted for the technical grade active ingredient and end-use product(s) and report on the product specifications. Product specifications include the active ingredient guarantee as well as information on type of formulation, the name and address of the manufacturer(s)/formulators of the technical grade active ingredient and end-use product(s) as well as a general discussion on the purpose of the individual formulation ingredients. If the toxicological characteristics of an ingredient in the formulation suggest a potential for human health or environmental hazard, a rationale should be submitted under DACO M2.9.1 as to why its use in the formulation is not considered to pose a significant risk.

Since the production of MPCAs is by nature a variable process, the variation in percent composition of the MPCA preparation, active ingredient guarantee and other formulation ingredients should be presented.

The applicant must provide MSDSs (Material Safety Data Sheet) or manufacturer's specifications, as well as related technical information, on all intentionally added ingredients listed on the SPSF such as emulsifiers, diluents, stabilizers or preservatives.

Part M2.9.2 Potency estimation and product guarantee

State the active ingredient guarantee (minimum) of the technical grade active ingredient and end-use product, expressed in recognized units of potency (for example, spores/mL; colony forming units [CFU]/kg) or other expression of biological activity per unit weight or volume such as International Toxic Units [ITU]/ mg.

For the MPCAs wherein potency units are not applicable, for example, 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, for example, percent germination and 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, it may be necessary to report the product guarantee and the MPCA content in more than one way. 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 M2.9.3 Unintentional ingredients

A discussion regarding the formation or presence of unintentional ingredients that are likely to occur in the particular MPCA preparation must be provided. Unintentional ingredients refer to impurities, contaminants or extraneous materials that are likely to occur in microbial products. These includes: 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.

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 end-use products or during any intermediary stage of production, data must be provided under Part M2.10.2 and M2.10.3 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, 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 end-use product (under Part M2.10.2 and M2.10.3).

Procedures designed to ensure purity or minimize levels of contaminants should be reported in Part M2.8, Manufacturing methods and quality assurance (see Section 6, Part M2.8). It may be possible to establish acceptable limits for some contaminants in consultation with PMRA officials. Rationales and supplementary information relevant to establishing limits of contaminants should be reported here.

Part M2.10 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 are required. The applicant should consult the PMRA regarding the nature of specific tests that may be required for certain types of MPCAs and products.

Part M2.10.1 Active ingredient or MPCA

Appropriate methodologies for enumeration and quantification of the entire microorganism, or parts thereof, are required in support of the active ingredient guarantee (in other words, potency). The methods must be described in detail, including standardization, sensitivity, reproducibility and statistical validity.

Representative potency data for five production batches of the technical grade active ingredient and from five production batches of the end-use product must be provided (and expressed in recognized units of potency) to confirm the guarantee reported under Part M2.9.1 and on the SPSF(s).

Depending on the nature of the microorganism appropriate methodologies for detection and isolation, and to distinguish the MPCA from other closely related strains may also be required. In certain cases, other methodologies may also be required such as monitoring for the active ingredient during production and quantification of doses for infectivity and toxicity testing, or enumeration of viable forms of the MPCA in tissues.

Note that methodologies for quantification or monitoring of metabolites, or specific chemical components, during production, belong under Part M2.10.3 rather than M2.10.1.

For 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 M2.10.2 Analysis for microbial contaminants

The levels of extraneous microorganisms in the technical grade active ingredient should not exceed those consistent with product safety and performance, as described in Section 6, Part M2.9.3 of this document. Suitable indicator organisms must be routinely monitored in production samples to assess the hygienic state of the production facility and process.

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.

All batches of technical grade active ingredient must conform to the limits set out in the Organisation for Economic Co-operation and Development (OECD) issue paper on microbial contaminants for microbial pest control products [ENV/JM/MONO(2011)43]. Based on information provided in Part M2.9.3, Unintentional Ingredients, it may be necessary to set limits for other microbial contaminants.

Claims concerning the nature and level of microbial contaminants must be supported by data from five production batches of technical grade active ingredient. As contaminant data is site-specific, representative batch data from each proposed manufacturing site of the technical grade active ingredient must be provided.

Details and validation of methods used to assay for microbial contamination must be submitted. Since the nature of microbial technical grade active ingredients 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 M2.10.3 Analysis for other unintentional ingredients

If a known toxic metabolite or hazardous substance may be present in a technical grade active ingredient or end-use product, precise and detailed methodologies for identification and analysis of the substance will be required.

If triggered based on the discussion in Part M2.9.3 and/or animal testing, submit methodologies and results of screens for any other potentially toxic unintentional ingredients, such as secondary metabolites/toxins. Include positive controls when analyses for secondary metabolites are required.

If any unintentional ingredients are expected to be produced in sufficient quantities to pose either a health or environmental concern, acceptable limits of metabolites/contaminants/impurities must be proposed. Refer to the *OECD Issue Paper on Microbial Contaminant Limits for Microbial Pest Control Products* [ENV/JM/MONO(2011)43] for additional guidance.

Part M2.11 Storage stability testing

The *Pest Control Products Act* and Regulations require that storage stability of products be verified to ensure product performance and safety for each technical grade active ingredient and each end-use product. 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, for example, suspendibility, wettability, viscosity;

- (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 end-use product and the technical grade active ingredient, if stored, should be tested over a suitable period of time and in accordance with typical storage and use conditions. Provide storage stability data from a suitable number of batches of technical grade active ingredient and end-use product (for example, five or more) to support the storage and shipping directions on the label.

For end-use products 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 M2.12 Summary of physical and chemical properties

The following physical and chemical properties, as applicable, for technical grade active ingredients and end-use products 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 M2.13 Characterization and analysis requirements for new end-use products of registered MPCAs

New end-use products of the currently registered MPCAs require submission of Part M2, Product characterization and analysis data in the following areas:

- Part M2.7 Characterization of the MPCA as necessary to validate equivalence of the MPCA to that first registered;

- Part M2.8 Manufacturing methods and quality assurance, relevant to the new end-use product and strain maintenance, as necessary to validate;
- Part M2.9 Disclosure of ingredients, product specifications, potency estimation and product guarantee, and unintentional ingredients, as relevant to the new end-use product;
- Part M2.10 Analytical data and methodology, as relevant to the new end-use product;
- Part M2.11 Storage stability testing, as relevant to the new end-use product; and,
- Part M2.12 Summary of physical and chemical properties, as relevant to the new end-use product.

Where procedures, manufacturing methods, quality control monitoring, etc., are similar to the original MPCA or end-use product submission, reference can be made to Part M2, Product characterization and analysis, of the original data package. Significant changes in manufacturing methods, quality control or analytical methodologies for technical and end-use products must be reported as a supplement to the original, Product characterization and analysis.

Overall, there must be assurance that the current MPCA does not differ from the registered MPCA. Part M2, 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 end-use products. Similarly, for new uses, additional safety data (in other words, Part M4, M9) 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 PMRA officials is recommended.

Part M3 Chemistry for registration of an end-use product or manufacturing concentrate

(not applicable to microbial end-use products)

Part M4 Human health and safety testing

a) Introduction

It is recommended that the characterization data requirements outlined in Section 6, Part M2.7, 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 6.0, Part M2.9.1, 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 6.0, Part M2.8, 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.

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 product characterization and analysis of the MPCA, 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 to testing

A complete evaluation of the product for human safety will be based both on the characterization data (Part M2) and on the data generated from human health and safety testing (Part M4). All available data on product safety should be submitted. Subsequent to the review of submitted data, however, additional data may be requested.

If the data from characterization and toxicology testing demonstrate no effects of concern, no further testing is required under Part M4. Where the data indicate otherwise, the approach for further testing depends on the use pattern of the product, 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 and registration will be denied. 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 under Part M4, as well as protocol recommendations, and minimum data requirements are summarized in the sections 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 6.0, Part M2.13, 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 for testing 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) Whenever possible, the same lot of test material should be used for all testing. 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) General conditions for human health and safety testing

Studies conducted under Part M4 must follow established test guidelines designed for microbial active ingredients, such as those outlined in [USEPA OCSPP Series 885](#).

The following test criteria should be considered for testing microbial pest control agents. 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 other words, pattern of clearance) 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 adequately establish a pattern of clearance;
- (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 guidelines, [Good Laboratory Practice](#). The document can be found on the [Pesticides section](#) of Canada.ca.

g) Waiver requests

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 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.

Where appropriate scientific data or rationales are available, the registrant is encouraged to request a waiver for data or endpoints that are not deemed to be relevant or essential. The requests to waive testing must be submitted as a sound scientific rationale with appropriate supporting information.

h) Human health and safety testing requirements

The human health and safety data requirements for MPCAs and their end-use products are outlined in Appendix II DACO Table for microbial pest control products. A summary is also provided in Appendix X.

Part M4.1 Summary

Part M4.2 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 M4.2.1 Summary

Part M4.2.2 Acute oral infectivity and toxicity

In an acute oral infectivity, toxicity and pathogenicity study (21-day) the technical grade active ingredient is administered as the test substance at a single high dose of at least 1.0×10^8 CFU/animal by oral gavage. If submitting an acute oral toxicity study, the pathogenicity potential of the MPCA must be addressed.

Part M4.2.3 Acute pulmonary infectivity and toxicity

In an acute pulmonary toxicity and pathogenicity study (21-day) the technical grade active ingredient is administered as the test substance at a dose of at least 1.0×10^8 CFU/animal by intratracheal instillation.

Part M4.3 Acute infectivity (IV or IP)

Part M4.3.1 Summary

Part M4.3.2 Intravenous infectivity (bacteria or viruses only)

In an acute infectivity study, a single high dose of the purest forms of the MPCA (at least 1.0×10^7 CFU/animal) is injected via the intravenous route.

Part M4.3.3 Intraperitoneal infectivity (fungi or protozoa only)

For fungi and protozoa, an acute infectivity study with a single high dose (at least 1.0×10^7 CFU/animal) of the purest forms of the MPCA is administered via intraperitoneal injection. If the intravenous route is possible, based on the physical characteristics of the microorganism, then it should be considered first.

Part M4.4 Acute dermal toxicity

In a dermal toxicity study on the end-use product, a single high dose is applied to approximately 10% of the body surface area of each test animal for a 24-hour exposure period. Alternatively, a combined study with dermal irritation can be conducted, provided the 24 hours exposure duration and irritation is scored according to standard guidelines for an irritation study.

Part M4.5 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.

MPCAs can also reasonably be expected to act as mild, reversible ocular irritants. Generally, this hazard can be adequately addressed through appropriate precautionary labelling, therefore, ocular irritation testing is not required. Based on the physical and chemical nature of the end-use product, standard hazard and precautionary statement will appear on the product label. Revisions to the label statements will only be considered based on eye irritation testing. An eye irritation study must be submitted under Part M4.9, Other Studies and Data.

If the applicant intends to modify the standard irritancy label hazard statements, irritancy data is required.

Part M4.5.1 Summary

Part M4.5.2 Dermal irritation study

A primary skin irritation study with the end-use product (or combined with M4.4/870.1200) is required if precautionary labelling is to be modified.

Acceptable protocol for dermal irritation testing include the *OECD Guidelines for Testing of Chemicals # A-404, Acute Dermal Irritation, 1993, Series 885.3100 Acute Dermal Toxicity/Pathology (Microbial Test Guidelines)* and *Series 870.1200 Acute Dermal Toxicity (Chemical Test Guidelines)*.

Part M4.6 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 MPCA formulations will therefore be considered potential sensitizing agents, and an appropriate label statement will be required.

However, 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 M4.7 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 M4.8 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 performance 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 6.0, Part M2.7.2, Biological properties of the MPCA(s) and Part M2.9.3, Unintentional ingredients. Suitable protocols for testing include the bacterial reverse mutation assay; mammalian erythrocyte micronucleus test, etc.

Part M4.9 Other studies and data

Part M4.9 is reserved to address additional human health and safety testing that does not correspond to a specific DACO. For example if an eye irritation study and acute oral toxicity study are conducted, the studies would be submitted under Part M4.9.

In particular, filamentous fungi are often formulated as dry fungal spore formulations, for example, wettable powder or solid formulations. Because these types of preparations pose health concerns from acute inhalation exposure, an acute toxicity study via the inhalation route is required for these products. This study would be submitted under Part M4.9. This is a unique requirement for certain types of MPCA preparations and is required in addition to the standard pulmonary toxicity and infectivity study (via the intratracheal instillation route of exposure) for the MPCA itself (Part M4.2.3).

Part M5 Exposure assessment

The information on the use pattern and potential for exposure to workers and bystanders provided under Part M1.2, Product profile and proposed use patterns, will be used to recommend protective clothing and decontamination procedures. Further exposure information under Part M5 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 M6 Metabolism/toxicokinetics studies (not applicable to microbial end-use products)

Part M7 Food and feed residue studies

Residue data are used to estimate the exposure of humans and livestock to potential residues of MPCAs that are proposed for use on food and feed crops, and to set and enforce the MRLs. As part of the assessment process prior to the registration of a pesticide, Health Canada must determine that the consumption of the maximum amount of residues that are expected to remain on food products when a pesticide is used according to label directions will not be a concern to human health. This maximum amount of residues expected is then legally specified as an MRL under the *Pest Control Products Act* for the purposes of adulteration provision of the *Food and Drugs Act*. Health Canada specifies science-based MRLs to ensure the food Canadians eat is safe.

When the characterization, in other words, Part M2, Product characterization and analysis, indicates the lack of potential for known mammalian toxin(s), and the acute oral toxicity test, in other words, Part M4.2.2, Acute oral infectivity and toxicity, shows that there will not be a significant human health concern, then the specification of an MRL, under the *Pest Control Products Act*, will not be required.

If the presence of a mammalian toxin has been identified and the applicant 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 (for example, uptake data; fate and persistence data).

Part M8 Environmental fate

a) Purpose

Environmental fate testing is intended to demonstrate whether an 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 environmental fate 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 (Part M9). The extent of environmental fate testing, if required for registration, is based mainly on the nature of the MPCA, in other words, whether it is indigenous or non-indigenous to the ecozone(s) of intended use.

Microbial pesticide ecozones of 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 (in other words, 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.

In contrast, 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 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, in other words, 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 environmental fate testing

For all MPCAs, an important consideration for field testing is protecting the environment in which exposure will occur. For this reason, field testing is only to be undertaken 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 6, Part M9, Environmental toxicology, section e) Tier Testing, and Appendix IX 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) Substance to be tested

The information required in these guidelines must be provided for the specific MPCA to be registered. However, it is recognized that information in published scientific literature and/or results in unpublished reports may provide much of the information required to meet the testing requirements for the MPCA. 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 M2, 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, in other words, pure active ingredient, is to be used for pure culture testing, while the end-use product is to be used for small- and large-scale field testing. For other fate tests, different forms of the MPCA, such as the technical grade active ingredient may be appropriate. The recommended form of the MPCA is specified in the testing requirements described below.

e) Tier testing (environmental fate)

If results of initial 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 based on use pattern.

f) Fate testing requirements

Part M8.1 Summary

Part M8.2 Laboratory studies

Laboratory studies are intended to determine:

- (i) the optimum and range of physical factors (for example, pH, temperature, light, oxidation- reduction potential, humidity, salinity, water activity) and osmotic pressure, and chemical factors (for example, 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, for example, 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 an 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.

Two types of Laboratory studies are discussed below in the next sections.

Part M8.2.1 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 M8.2.2 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 an MPCA to transfer to other organisms or to persist ex vivo in the environment.

To address the possible effects that additives in an end-use product may have on the fate of the MPCA, microcosm testing should be conducted with the end-use product. If it can be demonstrated that the additives intended for the end-use product 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 end-use product 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), in other words, 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 an MPCA or any introduced, genetic material in the case of a GEM. Some of these factors will be predetermined by the environmental medium, for example, soil type, soil pH, water hardness, salinity, while others can be controlled or managed, for example, temperature, soil moisture content, humidity, light intensity and quantity, oxygen concentration, water flow velocity. 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 [or: "...at a maximum challenge 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 (EEC) of the MPCA, immediately following a direct application at the maximum label rate to a 15-cm layer of soil or water;

Note: For both values use 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 M8.3 Greenhouse studies

As a bridge between laboratory testing and field studies, applicants may wish to conduct research trials under simulated outdoor conditions, in other words, 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 in support of a research permit may be required.

The need for data to support greenhouse studies 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 Part M8.4, Field studies, and in *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 M8.4 Field studies

As discussed above, field studies may be required for an MPCA that has a broad host range, in other words, 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 end-use product. Field studies should address such aspects of environmental fate as:

- (i) growth or decline (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 *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 M9 Environmental toxicology

a) Purpose

Part M9 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, in other words, formulants, in end-use products, 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 environmental toxicology testing

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 is discussed in detail under section e) and the tier testing scheme is outlined in Appendix VIII, Environmental toxicology and environmental fate testing tiers.

The extent of the environmental toxicology testing requirements at each tier is dependent on whether the MPCA is indigenous or non-indigenous to the ecozone of intended use. 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.

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 definitive toxicity testing, for example LC₅₀, LD₅₀, as well as chronic toxicity, in other words, life cycle testing. 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.

The Tier II data determines 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 (for example, definitive toxicity testing; chronic toxicity testing) is reserved only for non-indigenous MPCAs that produce significant adverse effects on non-targets in Tier II.

c) Considerations for extent of environmental toxicology testing

When an MPCA is applied, large numbers of the MPCA 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, for example, 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 end-use products containing GEMs are similar to those required for MPCAs containing non-indigenous microorganisms as defined in Part M8, 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, in other words, 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.

Appendix X Summary of non-target toxicology testing provides guidance on selecting non-target organisms for environment toxicology testing, along with Appendix XI and Appendix XII for non-target arthropods and plants, respectively.

The applicant is encouraged to prepare a strategy for non-target testing based on this information and in line with the advice in the Appendix II DACO table for microbial pest control products. Once a strategy has been developed, a pre-submission consultation can be arranged to address specific concerns and questions.

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, (in other words, phenotypic or genotypic), or phylogenetic, (in other words, 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, as the target organism.

Host range studies undertaken by the applicant to fulfil efficacy data requirements in Part M10, Value assessment (see Section 6, Part M10), may, in some cases, be suitable to support the initial non-target organism testing requirements.

For genetically modified microorganisms, in other words, MPCAs modified through classical genetic techniques such as chemical mutagenesis, 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, in other words, 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, for example, species for which the affected target host is 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 f) 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 maybe toxic but 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 *Good Laboratory Practice*.

- (iv) **Substance to be tested:** Because MPCAs can exist in more than one form, consideration must be given to the most suitable form and purity of the MPCA for testing. 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 M2, 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 end-use product 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 end-use product may not always be feasible. Therefore, the technical grade active ingredient of the MPCA can be employed in Tier I, II and III tests. In addition, any substances, in other words, adjuvants or formulants, intended to enhance the virulence or toxicity of the MPCA should be tested along with the technical grade active ingredient. In Tier IV actual or simulated field trials, only the end-use product should be used. If the technical grade active ingredient of the MPCA and end-use product are identical, however, Tier IV testing may also be carried out with the technical grade active ingredient 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 organisms 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, for example, bacterium, fungus, 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 technical grade active ingredient (for details see Section M9.2, section ii).

In most cases, testing at one concentration, in other words, the MCC, is expected to be sufficient to evaluate adverse effects. Consequently, if no adverse effects are observed at the MCC (Tier I), testing at lower concentrations (Tier II) 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, in other words, 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, in other words, positive controls. Alternatively, the activity of the MPCA, in terms of viability, can be assessed by another technique, for example, 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 end-use product 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, in other words, the 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, for example, formulants, adjuvants, intended for the end-use

product 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 in lieu of testing:** The data requirements specified in Section 6, Part M9, Environmental toxicology, apply to a wide variety of MPCAs with diverse biological properties, for example, viruses, bacteria, fungi, protozoa, algae, 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 end-use product to be registered, an applicant can request a waiver for that requirement. To request a waiver, the applicant must submit a sound scientific rationale in lieu of a study. 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, and, when appropriate, suggest alternative means of obtaining data to address the concern that underlies the data requirement. The waiver rationale must be supported by published literature search on toxicity/pathogenicity of the MPCA, and/or closely related microorganisms, to support claims of low risk to the specific non-target group (birds, fish, etc.). Environmental fate/expression data, for example, maximum growth temperatures; growth requirements, may also be used to support the scientific rationale if claims of low risk are based on an expected low level of exposure to the MPCA under operational conditions of use, for example, during application and postapplication of the end-use product, or if MPCA cannot grow at host body temperature. 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 by use pattern* and summarized in Appendix X, *Summary of the test substance for non-target toxicology testing*.

The general strategy for testing each of the major non-target organism groups is specified in section f) 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. And if the MPCA is non-indigenous to the ecozone(s) of intended use, environmental fate (see Section 6, Part M8, 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.

- (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, for example, honeybees, may be sufficient to trigger Tier II testing. In other cases where adverse effects are observed on non-target species that are of lesser importance, it may take a number of non-target species 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 for Tier II testing is specified in each testing requirement and, in most cases, is a lower challenge concentration (LCC) than the MCC used in Tier I testing. In some cases, Tier II testing will represent a different route of

exposure, rather than a change in test concentration. For example, if Tier I toxicity testing was conducted via dietary exposure at the MCC, Tier II testing may be conducted by intravenous or intraperitoneal injection at the MCC. Tier II testing will not be required when the route of exposure and challenge concentration are expected to be the same as those used in Tier I.

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 Part M8, 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 M8) 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. Because an MPCA that produces significant adverse effects on non-target species in Tier II tests could pose an unacceptable risk, it is imperative that Tier III and Tier IV tests not be conducted unless specifically requested, following the initial review of Tier I and Tier II test results.

If Tier III testing is required, 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., LC₅₀, 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, in other words, 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 LD₅₀ or LC₅₀, defined as the dose or concentration required to kill 50% of the test organisms, or the ED₅₀ or EC₅₀, 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 LD₅₀, LC₅₀, ED₅₀ or EC₅₀ 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 LD₅₀, LC₅₀, ED₅₀ or EC₅₀ 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 LD₅₀, LC₅₀, ED₅₀ or EC₅₀ 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.

Indigenous MPCAs are exempt from toxicology testing at Tier III even if adverse effects are noted on test organisms in Tier II.

- (iv) **Tier IV:** If adverse effects are produced on non-target organisms in Tier II, Tier IV simulated or actual field testing of indigenous and non-indigenous MPCAs is required. 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 end-use product 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 M9.1 Summary

Part M9.2 Birds

Non-target avian species are likely to be exposed to the MPCA most commonly through the oral route via feeding of infected food, for example, insects, or seeds, 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.

(i) Test species (avian)

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. For agricultural uses in particular, the bobwhite quail is preferred. 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 LD₅₀) 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 avian species

For Tier I tests, the MCC should be a function of the concentration of the MPCA in the technical grade active ingredient and the route of administration.

- Oral = $\text{MPCA Concentration in technical grade active ingredient (units MPCA/mL)} \times 5.0 \text{ mL/kg Body Weight} \times \text{Weight of Bird (kg)}$
- Pulmonary = $\text{MPCA Concentration in technical grade active ingredient (units MPCA/mL)} \times 0.2 \text{ mL/kg Bird Body Weight} \times \text{Weight of Bird (kg)}$
- Intravenous= $\text{MPCA Concentration in technical grade active ingredient (units MPCA/mL)} \times 0.5 \text{ mL/kg Bird Body Weight} \times \text{Weight of Bird (kg)}$
- Intraperiton= $\text{MPCA Concentration in technical grade active ingredient (units MPCA/mL)} \times 2.0 \text{ mL/kg Bird Body Weight} \times \text{Weight of Bird (kg)}$

If the maximum dose tested in Tier I studies is lower than the MCC, a justification must be provided.

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

(iii) Testing requirements

Part M9.2.1 Avian oral

The Tier I data requirement for avian oral testing is a 21-day avian oral study using the technical grade active ingredient as the test substance, administered by gavage at the MCC, daily for five consecutive days.

Part M9.2.2 Avian pulmonary, inhalation or injection

Tier I data requirements for acute pulmonary testing is an intratracheal instillation study at the MCC, daily for five successive days.

Depending on the physical properties of the technical grade active ingredient being tested, aerosolization may be used as an alternate to pulmonary exposure provided that a written justification for choosing this method be submitted to the PMRA. Pulmonary data conducted using the alternative inhalation, in other words, aerosolization, method should also expose birds to the MCC daily for five consecutive days.

An injection, in other words, 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 defense mechanisms. 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.

Tier testing for avian species

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 an 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 M9.3 Wild mammals

The toxicology data required in Part M4, 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 Part M4, 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 Part M4, 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 Part M4, 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, in other words, Part M4, Human health and safety testing, 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 M4, Human health and safety testing, additional testing may be required. For MPCAs or end-use products 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 M9.4 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 (aquatic exposure); and/or (2) diet, either in the form of diseased target pests (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 end-use product or in amended artificial feed stock.

For Tiers I, II and III (definitive LC₅₀, EC₅₀) 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 (fish)

For 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.

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

If the MPCA is intended to control nuisance fish, for example, 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 under Part 9 Environmental toxicology, Section d General information, Section ii) Selection of non-target organisms and take into account species that occur in drainage basins in the areas (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) for fish species

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 EEC of the MPCA, immediately following a direct application at the maximum label rate to a 15-cm layer of water

Note: For both values use 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 by-products 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 (active units of the MPCA per gram of feed) should be equivalent to the maximum concentration found in the target (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 end-use product, if these additives are intended to enhance virulence or toxicity of the MPCA.

(iii) Testing requirements for fish species: The testing requirements for freshwater fish and estuarine/marine fish species are discussed below.

Part M9.4.1 Freshwater fish

The Tier I data requirement for freshwater fish testing is a 30-day static-renewal freshwater fish study using the technical grade active ingredient as the test substance 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.

One cold water fish species, preferably rainbow trout (*Oncorhynchus mykiss*). 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. Additional testing may be required on bluegill sunfish (*Lepomis macrochirus*) if there is potential for significant freshwater exposure.

Part M9.4.2 Estuarine or marine fish

Tier I testing of estuarine or marine fish species is conditionally required in cases where exposure to estuarine or marine environments is likely to occur and the MPCA is capable of surviving in saline water. 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.

(iii) Tier testing

If Tier I testing indicates a hazard to freshwater or estuarine/marine fish species, appropriate information from Tier II may be required. If Tier II environmental toxicology testing is required for fish, 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 LC₅₀ or EC₅₀ 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 M9.5 Arthropods (terrestrial and aquatic)

Tier I testing of terrestrial and aquatic non-target species is required. In testing the effects of the MPCA on terrestrial and aquatic arthropod species, 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.

For terrestrial arthropods, the MPCA should be administered:

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

For aquatic arthropods, the MPCA should be administered:

- as a suspension in the water, in other words, 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 (terrestrial arthropods)

Because of their critical role in environmental sustainability, honeybee testing (*Apis mellifera* (L.); *Hymenoptera*) is mandatory. Other 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 6, Part M9 Environmental toxicology, section d General information, Part 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, insects used for biological control, for example, *Apanteles* spp. (*Hymenoptera*), predatory orchard mites, for example, *Neoseiulus fallacis* (Garman) (Acari), *Phytoseiulus persimilis* (Acari).

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, for example, *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: Whenever possible, effects testing should be conducted on representatives of the terrestrial non-target arthropod taxa listed in Appendix XI. Each of the taxa listed contains environmentally or economically important species that may be obtained from Canadian arthropod culture collections.

Generally, toxicity/pathogenicity testing is required using the technical grade active ingredient on at least three species of representative arthropods representing at least two of the following groups – parasitic dipterans, predaceous hemipterans, predaceous coleopterans, predaceous mites,

predaceous neuropterans, parasitic hymenopterans. Exposure data (likelihood) or intended use pattern(s) may preclude non-target insect testing, and depend on adequate host range/identification of the MPCA.

For MPCAs applied in aquatic use patterns, or applied in terrestrial use patterns where aquatic exposure is anticipated: Effects 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. Typically, *Daphnia magna* and *Daphnia pulex* (Cladocera) are selected as representative freshwater aquatic arthropods.

(ii) Maximum challenge concentration (MCC) for arthropod species

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 EEC of the MPCA, immediately following a direct application at the maximum label rate to a 15-cm layer of soil or water;

Note: For both values use 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 by-products 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 (active units of the MPCA per gram of feed) should be equivalent to the maximum concentration found in the target (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 end-use product, if these additives are intended to enhance virulence or toxicity of the MPCA.

(iii) Testing requirements for arthropod species

Because of their critical role in environmental sustainability, toxicity/pathogenicity testing using the technical grade active ingredient on honeybee is required. OCSPP Guideline 885.4380 recommends an exposure/observation period of 30 days. Since the OECD guidelines are used to test acute hazards/exposures to chemical pesticides, they are not appropriate for testing potential hazards of the MPCA to honeybees, as they do not include extended periods of duration.

In addition, toxicity/pathogenicity testing required using the technical grade active ingredient on at least three species of representative arthropods are also required, as discussed above.

For Tier I topical exposure tests, non-target arthropods should be treated with the MCC for five successive days and then observed for an additional 16 days.

For Tier I testing, non-target arthropods should be exposed to, or fed the MCC of the MPCA or end-use product for at least 21 days, or until mortality in the control group increases to a significant level.

(iv) Tier testing

If Tier I testing indicates a hazard to arthropod species, appropriate information from Tier II may be required. 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.

If Tier III testing is triggered, definitive toxicity testing to establish the LC_{50} or EC_{50} of the MPCA will be required. Although Tier III life cycle testing may also 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.

Indigenous MPCAs are exempt from Tier III toxicology testing, even if adverse effects are observed at the Tier II level, but fate testing may be required at Tier III, if triggered by the results of Tier II toxicology testing.

For indigenous and non-indigenous MPCAs, simulated or actual field testing of susceptible non-target arthropods may be necessary at Tier IV 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 M9.6 Non-arthropod invertebrates

Tier I testing of non-arthropod invertebrates is conditionally required in cases where the MPCA is intended to control invertebrates, such as annelids (for example, earthworms; *Lumbricus terrestris*, or molluscs). In selecting appropriate non-arthropod invertebrates for effects testing with the MPCA, applicants should follow the centrifugal taxonomic approach as outlined in Section 6, Part M9 Environmental toxicology, section d General information, 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 and outlined in Appendix XI Suggested taxa for selection of non-target arthropods.

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 M9.7 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 (microorganisms)

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, in other words, 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) for microorganisms

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;

Note: For both values, use whichever is greater or achievable.

(iii) Testing requirements for microorganisms

Tier I testing of microorganisms 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 M8, Environmental fate, 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, but may be warranted if long-term and irreversible adverse effects on non-target microorganisms, or processes of environmental or economic importance, are demonstrated from the Tier I data. 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 M9.8 Plants

MPCAs intended to control pest plants, in other words, herbicidal MPCAs, and any end-use products 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, in other words, 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 end-use product 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 (terrestrial and aquatic non-target plants)

Selection of non-target plant species should take into account such factors as the purpose of the MPCA, in other words, 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 non-target plant species to any target pest species. For additional guidance in selecting appropriate non-target plant species, refer to Section 6, Part M9 environmental toxicology, section d General information, part ii) Selection of non-target organisms. Applicants should also endeavour to test some weed and wild plant species.

Ultimately, selection of non-target plant species depends on whether the intended use pattern of the MPCA is aquatic or terrestrial, with specific considerations given to terrestrial forestry uses.

Specific considerations for selecting appropriate non-target terrestrial plant species and aquatic plant species are discussed below.

Part M9.8.1 Terrestrial plants

The following four scenarios should be considered when selecting the appropriate non-target terrestrial plant species for testing.

For MPCAs intended for terrestrial use, where direct aquatic exposure is not anticipated: Non-target terrestrial plant species for testing should be selected from the representative terrestrial plant families listed in Appendix XII, Suggested taxa for selection of non-target plant species based on use pattern. 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 specifically for forestry use and for which direct aquatic exposure is therefore anticipated: As listed in Appendix XII, non-target terrestrial plant species for testing should include species of Pinaceae. Furthermore, aquatic plants species should also be selected for testing, as discussed further under Part M9.8.2.

For non-herbicidal MPCAs that are taxonomically related to known phytopathogenic microorganisms: Certain non-herbicidal MPCAs may be taxonomically related to a known phytopathogenic microorganisms, and as such, there is a concern that the MPCA may also display phytotoxicity. In such a case, plant species known to be susceptible to the related pathogen should be selected as a starting point to assess the phytopathogenicity of the MPCA. If pathogenic effects are noted in any of these non-target plants species, further testing utilizing the centrifugal taxonomic approach would be required with the affected plant species as the new starting point.

For MPCAs intended for direct aquatic applications (in other words, to control aquatic plants) and for which terrestrial exposure is not anticipated: Effects testing on non-target terrestrial plants will not ordinarily be required. However, if terrestrial exposure is anticipated from the aquatic use pattern, for example, via spray drift from aerial applications, then non-target terrestrial plant testing may be required.

Part M9.8.2 Aquatic plants

For MPCAs intended for aquatic use or for terrestrial use where aquatic exposure is anticipated: Testing should be performed on aquatic plant species selected from up to six representative aquatic plant families listed in Appendix XII, Selected taxa for selection of non-target plant species, based on the most relevant species for the anticipated use pattern. 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.

For MPCAs intended for forestry applications and for which aquatic exposure is expected to occur: Effects testing on aquatic non-target plants may be required. Testing should be performed on aquatic plant species selected from representative aquatic plant families listed in Appendix XII, Selected taxa for selection of non-target plant species, based on the most relevant species for the anticipated use pattern.

For MPCAs intended for terrestrial uses, where direct aquatic exposure is not anticipated, or the MPCA is not expected to survive in aquatic environments: Effects testing on non-target aquatic plants will not ordinarily be required.

(ii) Maximum challenge concentration for terrestrial and aquatic non-target plants

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;

Note: For both values, use whichever is greater or achievable.

(iii) Testing Requirements for non-target plants (terrestrial and aquatic)

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, in other words, seed emergence and root elongation testing, and young vegetative growth, in other words, vegetative growth and vigour testing, will be required. Plants should be tested with a concentration of the MPCA or end-use product that is equivalent to the maximum rate of application proposed on the product label.

Tier III toxicology 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 toxicology testing.

Tier IV testing should be conducted on small-scale field plots with the end-use product 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 M10 Value assessment (including efficacy)

a) Introduction

Consistent with the requirements of the *Pest Control Products Act* and Regulations and the mandate of the PMRA, assessment of the value of the end-use product 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) end-use product performance, according to prescribed label conditions and claims;
- (iii) current management tools: status, benefits, problems; and
- (iv) the contribution of the end-use product 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 end-use product to sustainable crop and pest management or risk reduction with possible drawbacks or disadvantages. It is anticipated that end-use products 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 end-use product 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 end-use product 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, for example, forestry, agriculture, field data from relevant regions in the United States 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, for example, 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, for example, optimum methods and timing of application, host or pest phenological requirements and constraints, population density, environmental factors, are required. Proponents should consult *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, *Guidelines for Efficacy Assessment of Fungicides, Bactericides and Nematicides*, and competing vegetation management, *Guidelines for Efficacy Assessment of Herbicides and Plant Growth Regulators*, should also be consulted, as applicable to the end-use product.



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 end-use products. 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 end-use product applications per season.

For this reason, the fundamental principles of efficacy testing developed for traditional control products are considered to be applicable to many end-use products. 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 *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 M10.1 Summary

Part M10.2 Performance assessment

Part M10.2.1 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, for example, 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 end-use product.

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 LC₅₀, LC₉₅, LT₅₀, 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 technical grade active ingredient or formulation intermediate 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 end-use product 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, in other words, 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 end-use product 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 M10.2.2 Field studies

For the purpose of this guideline, field studies refer to testing of the proposed end-use product 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 end-use product 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 end-use product 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 end-use product field efficacy trials are summarized below. Proponents should also consult Section 2, *Guidelines for Efficacy Assessment of Chemical Pesticides*, for a more detailed explanation.

- (i) Performance testing should be conducted using the end-use product 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 end-use product 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 end-use product 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 end-use product 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 end-use product 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, for example, 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 end-use products, 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 end-use product 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:
 - C only a limited, regional registration is desired; or
 - C 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 end-use product with adjuvants, stickers, spreaders, diluents, etc., should be supported by compatibility and performance data.

Part M10.3 Treatment effects

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

Part M10.3.1 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 end-use products containing MPCAs with potential for plant injury, in other words, 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 end-use products, 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, for example, seeds, cuttings, runners, should be reported.

Part M10.3.2 Compatibility with crop protection and management practices

Part M10.3.2.1. Effects on MPCA performance

Information on the efficacy and compatibility of the end-use product 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 end-use product performance. These and any other crop management practice that may negatively affect performance should be evaluated, where relevant to the nature of the end-use product and the production scenario.

Part M10.3.2.2. Effects of the end-use product

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, in other words, Part M10.2, Performance assessment (see Section 6, Part 10.2), and relevant non-target hazard testing data, in other words, Part M9, Environmental toxicology (see Section 6, Part M9), 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 M10.4 Crop or resource production benefits

To document the potential value of a new end-use product 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 end-use product.

New end-use products 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 end-use products 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, for example, 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, for example, 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, for example, corn tasselling, indoor uses, aerial application in residential areas.

Part M10.4.1 Profile of the end-use product

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

Part M10.4.2 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 United States where relevant. The information should be presented in the context of the proposed use pattern and anticipated impact of the end-use product on the problem.

Most end-use products, 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 end-use product 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 *Assessment of the Economic Benefits of Pesticides*, for guidance.

Part M10.4.3 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 end-use product proposed for registration.

This information should be presented in the context of unique performance or safety features of the end-use product that may complement benefits or mitigate drawbacks of current tools, for example, 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 end-use product, for example, crop rotation, zero till, soilless cultivation.

Part M10.4.3.1. Contribution to integrated pest management strategies and practices

End-use products may exhibit features, for example, relatively narrow host range or limited environmental persistence, that constitute a key element in the development and implementation of IPM strategies. Based on the end-use product profile, host range and reported performance data, the actual or potential role of the new end-use product 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 end-use product 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 end-use product 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, for example, *Bacillus thuringiensis* (Bt) products, use recommendations should include advice regarding measures to mitigate resistance development.

In the case of end-use products 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, for example, Bt plants, strategies for integrated use of the end-use product and the resistant plants should be outlined.

Part M11 Not applicable to microbial pest control products

Part M12 Summaries

Part M12.5 Foreign reviews

Foreign reviews should be coded as Part M12.5.x (DACO Part Number) and included at the end of the applicable Part. For example, USEPA Reviews of Toxicology would be coded M12.5.4 and included at the end of the information provided for Part M4, Human health and safety testing.

Part M12.7 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 if available. 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 *Comprehensive Data Summaries*. The general format of the summaries should follow those described in the European Commission (EC) guidelines, in other words, based on the EC Guideline 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.

Appendices

Appendix I Glossary

Acronyms

CR	conditionally required
DACO	data code
DNA	deoxyribonucleic acid
EC	European Commission
EC ₅₀	effective concentration 50%; the concentration that effects 50% of the population
ED ₅₀	effective dose 50%; the dose that effects 50% of the population
EEC	expected environmental concentration
Eh	redox potential
ELISA	enzyme linked immunosorbant assay
EU	European Union
FDA	<i>Food and Drugs Act</i>
GEM	genetically engineered microorganism
GLP	Good Laboratory Practice
ha	hectare(s)
HC	Health Canada
HPLC	high performance 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	lethal concentration 50%; the concentration that kills 50% of the population
LC ₉₅	lethal concentration 95%; the concentration that kills 95% of the population
LD ₅₀	lethal dose 50%; the dose that kills 50% of the population
LT ₅₀	lethal time 50%; the time for 50% of the population to die
MAR	maximum application rate
MCC	maximum challenge concentration
MA	manufacturing concentrate
MPCA	microbial pest control agent
MRL	maximum residue limit
MSDS	Material Safety Data Sheet
OECD	Organisation for Economic Co-operation and Development

PCPA	<i>Pest Control Products Act</i>
PMRA	Pest Management Regulatory Agency
QA	quality assurance
QC	quality control
R	required
SHE/SAV7	primary Syrian hamster embryo system
USEPA	United States Environmental Protection Agency

Terms



These definitions explain how terms are used in this document. If there is a conflict with a definition in the *Food and Drugs Act* or associated regulations, the definition in the Act or regulations prevails.

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 II, Data Code (DACO) Table for Microbial Pest Control Products, for a complete list of DACOs for microbials. This code is a requirement of the *Pest Control Products Act* and PCPR.

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 – A formulation intended for pest control purposes which includes labelling with specific 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.

Microbial end-use products specifically refer to end-use formulations that contain a microbial pest control agent as the active ingredient (rather than a chemical active ingredient).

Manufacturing concentrate – A preparation containing the technical grade active ingredient (microbial or other) to which other ingredients have been added, for example, preservatives, stabilizers, diluents, to produce a preparation suitable for use in the manufacture of an end-use product. Manufacturing concentrates can be prepared with microbial or conventional chemical active ingredients.

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 – In the context of MPCAs, an indigenous MPCA is a strain that has been 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 – In the context of microbial pest control products, residues refer to the number of microbial organisms (or identifiable parts thereof) that are left on the crop following application. Residues may also refer to a measurable quantity of a representative chemical component or metabolic by-product of the MPCA.

Technical grade of the active ingredient – 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 technical grade active ingredient 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 a manufacturing concentrate or end-use product.

Specifically, microbial technical grade active ingredients contain a microbial pest control agent as the active ingredient whereas conventional technical grade active ingredients contain a chemical as the active ingredient.

Tiering – A multilevel or tier system of information requirements is a feature of the approach to environmental testing, in other words, Part M8, Environmental fate and Part M9, Environmental toxicology; (see Appendix VIII) for more details. Initial submissions must include results for all required Tier I tests. 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.

Appendix II Data code (DACO) table for microbial pest control products

Data Code (Part)	USEPA OCSPP Guideline Number	Title	Data Required ¹	Additional Comments or Conditions ²
M0 INDEX			R	
M1 LABEL/PRODUCT SUMMARY AND USE PATTERNS/INTERNATIONAL REGULATORY STATUS				
1.1.1	40CFR 152.50	Label	R	
1.1.2	40CFR 156			
M1.2	40CFR 158.2120 40CFR 152.50(e) 40CFR 156	Product profile and proposed use patterns	R	
M1.3		International regulatory status of the MPCA and end-use product	R	
M2 PRODUCT CHARACTERIZATION AND ANALYSIS				
M2.1	40CFR 152.50	Name and address of applicant	R	
M2.2	40CFR 167.20	Name and address of manufacturing plant	R	
M2.3	40CFR 167.20	Name and address of formulating plant (if different from M2.2)	R	
M2.4	885.1100	Trade name	R	
M2.5	885.1100	Binomial name (MPCA)	R	
M2.6		Canadian patent status information	R	
M2.7 Characterization of the MPCA / Product identity				
M2.7.1	885.1100	Origin, derivation, and identification of MPCA(s)	R	Provide details on the origin, preservation and maintenance of the MPCA including a morphological description; conventional and molecular tests to establish identity and describe all methods used to distinguish the MPCA from other strains or closely-related genera/species. List all alternatives/synonyms and/or superseded names associated with the MPCA.

Data Code (Part)	USEPA OCSPP Guideline Number	Title	Data Requirement ¹	Additional Comments or Conditions ²
M2.7.2	885.1100	Biological properties of the MPCA(s)	R	Detailed information is required on the biological properties of the MPCA with an emphasis on any potentially hazardous biological properties. Discuss natural occurrence; target organisms; effects on species closely related; and history of its use (for agricultural, forestry or therapeutic uses), physiological properties (for example, growth parameters; nutritional dependence) and susceptibility/tolerance to anti-microbial agents used in human/veterinary medicine. Describe the mode of action of the MPCA, including mechanisms of pathogenicity/toxicity, and any secondary metabolites produced by this strain. Discuss the relationship of the MPCA to known pathogenic/toxigenic strains or species, and any adverse effects related to human exposure for this species. A detailed literature search (with keywords, databases) including pertinent literature in full text form, may be appropriate to address this information. Refer to Part M2.7.1 in DIR2001-02 for more details.
M2.7.3 Characterization of MPCAs derived through recombinant nucleic acid technologies				
M2.7.3.1		Taxonomy and characterization of recipient and donor microorganisms	CR	Required if the MPCA was derived through recombinant nucleic acid technologies.
M2.7.3.2		Construction of the recombinant microorganism	CR	Required if the MPCA was derived through recombinant nucleic acid technologies.
M2.7.3.3		Nature and expression of introduced or modified genetic material	CR	Required if the MPCA was derived through recombinant nucleic acid technologies.
M2.7.3.4		Phenotypic characterization of the modified microorganism	CR	Required if the MPCA was derived through recombinant nucleic acid technologies.

Data Code (Part)	USEPA OCSPP Guideline Number	Title	Data Required ¹	Additional Comments or Conditions ²
M2.8 Manufacturing methods and quality assurance				
M2.8	885.1200 885.1250 885.1300 885.1400 885.1500	(a) Preservation and maintenance of the production strain (b) Manufacturing process (c) Quality assurance Deposition in a culture collection	R	Provide a description of the manufacturing process (for example, preservation, maintenance of the production strain, culture media components; preparation procedures, scale-up, commercial scale cultivation, harvest and process of the final culture, formulation methods, packaging, storing). A detailed description of the manufacturing facility (instrumentation) and the quality assurance program (cleaning, sterilization, quality control procedures) and tests for identification and quantification of contamination must also be provided. A sample of the MPCA must be deposited in an internationally recognized culture collection.
M2.9 Disclosure of ingredients / Certification of limits				
M2.9.1	885.1100,885.1300 885.1400885.1500 40 CFR 158.350(a)(3)	Product specifications	R	Submit an SPSF for the technical grade active ingredient and each end-use product with the active ingredient guarantee expressed in appropriate units (in other words, CFU/g). The CAS numbers for each inert/formulant ingredient and any impurity of toxicological significance should have an upper limit designated as a certified limit on the SPSF.
M2.9.2	885.1100	Potency estimation and product guarantee	R	State the active ingredient guarantee of the technical grade active ingredient and end-use product, supported by data for 5 batches from each production/formulating site (submitted under M2.10.1). All data must be specific to the proposed manufacturing site(s) of the technical grade active ingredient; if multiple formulating sites are used, representative data are required from each site. If possible, correlate the concentration of each MPCA with biological activity against the targeted pest. Discuss any production efforts employed to limit batch-to-batch variability.
M2.9.3	885.1200885.1300 885.1500	Unintentional ingredients	R	Provide a discussion on the potential for impurities (for example, microbiological contaminants, allergens, metabolites; as identified in M2.7.2, M2.8) and the impact on product quality.

Data Code (Part)	USEPA OCSPP Guideline Number	Title	Data Requirement ¹	Additional Comments or Conditions ²
M2.10 Analytical data and methodology / Analysis of samples				
M2.10.1	885.1300	Active ingredient or MPCA	R	Provide detailed method protocols (with validation) for enumeration of the MPCA, in support of the active ingredient guarantee (in other words, potency data). Representative potency data for five production batches of the technical grade active ingredient and five production batches of the end-use product must be provided. As needed, also provide methods for detection, isolation of the MPCA and to distinguish the strain from other strains or phylogenetically close genera/species. Methods for quantification of doses for infectivity and toxicity testing, or enumeration of viable forms of the MPCA in tissues may also be required.
M2.10.2	885.1300 885.1400 885.1500	Analysis for microbial contaminants	R	Provide detailed screening methods, using selective (in other words, microbe-specific) media, following OECD (ENV/JM/MONO(2011)43) or other internationally recognized protocols. Acceptable limits, as listed in OECD [ENV/JM/MONO(2011)43], should be proposed, if expected to be in sufficient quantities to pose either a health or environmental concern. Submit microbial contaminant data for at least five batches of technical grade active ingredient, specific to the proposed manufacturing site(s).
M2.10.3		Analysis for other unintentional ingredients	CR	If triggered under M2.9.3 and/or Tier I animal testing (M4), submit protocols and results from five batches of screens for any other potentially toxic unintentional ingredients, for example, toxic secondary metabolites. Include positive controls, when required. Acceptable limits of metabolites/contaminants/impurities must be proposed. Refer to the "OECD Issue Paper on Microbial Contaminant Limits for Microbial Pest Control Products" [ENV/JM/MONO(2011)43] for additional guidance.
M2.11	885.1400 830.6317	Storage stability testing	R	For representative batches of the technical grade active ingredient (if exists as a separate product) and end-use product at the proposed storage temperature(s) indicated on the label. A range of temperatures and multiple sampling times are recommended.

Data Code (Part)	USEPA OCSPP Guideline Number	Title	Data Required ¹	Additional Comments or Conditions ²
M2.12	40CFR 158.2120(c)	Summary of physical and chemical properties	R	For the technical grade active ingredient and end-use product. Include colour, physical state; odour, stability, miscibility, pH, density, bulk density or specific gravity; viscosity, and corrosion characteristics and methods or cite published methods.
M2.13		Characterization and analysis requirements for new end-use products and registered MPCAs	NR	
M2.14		Other studies/data	CR	Required if other studies/data are available.
M4 HUMAN HEALTH AND SAFETY / TOXICOLOGY TESTING³				
M4.1		Summary	R	
M4.2 Infectivity, pathogenicity and toxicity				
M4.2.1		Summary	R	
M4.2.2	885.3050	Acute oral infectivity and toxicity	R	Submit an acute oral infectivity, toxicity and pathogenicity study (21-day) using the technical grade active ingredient(s) ⁴ as the test substance. Administer test substance by gavage at a single high dose of at least 1.0×10^8 CFU/animal. If submitting an acute oral toxicity study, the pathogenicity potential of the MPCA must be addressed.
M4.2.3	885.3150	Acute pulmonary infectivity and toxicity	R	Submit an acute pulmonary, toxicity and pathogenicity studies (21-day) using the technical grade active ingredient(s) as the test substance. In order to assess pathogenicity, a dose at least 1.0×10^8 CFU/animal administered by intratracheal instillation must be achieved.
M4.3 Acute injection infectivity/pathogenicity (IV or IP)				
M4.3.1	40 CFR 152.2140(c)	Summary	R	
M4.3.2	885.3200	Intravenous (IV) infectivity (bacteria or viruses)	R	Submit an acute infectivity study using a single high dose of the purest forms of the MPCA, ⁵ injected via the intravenous route. A minimum dose of 1.0×10^7 CFU/animal is required.
M4.3.3	885.3200	Intraperitoneal (IP) infectivity (fungi or protozoa)	R	Submit an acute infectivity study using a single high dose of the purest forms of the MPCA, ⁵ injected via the intraperitoneal route. A minimum dose of 1.0×10^7 CFU/animal is required.

Data Code (Part)	USEPA OCSPP Guideline Number	Title	Data Required ¹	Additional Comments or Conditions ²
M4.4	870.1200	Acute dermal toxicity	R	Submit a dermal toxicity study on the end-use product ⁶ or combine with M4.5.2/870.2500 (dermal irritation), provided the 24 hours exposure duration and irritation is scored according to standard guidelines for an irritation study.
M4.5 Irritation				
M4.5.1		Summary	R	
M4.5.2	870.2500	Dermal irritation study	R	Submit a primary skin irritation study for the end-use product (or see M4.4/870.1200).
M4.6	40 CFR 158.2140(c)	Reporting of hypersensitivity incidence	R	No hypersensitivity testing is required, but any recent incidents of adverse effects from manufacturing, handling or application of the MPCA must be reported.
M4.7	885.3500	Tissue culture (viral agents only)	NA	
M4.8		Genotoxic potential	CR	If the MPCA can produce potentially genotoxic metabolites (M2.7.2 and M2.9.3.), the genotoxic potential of these technical grade active ingredients must be characterized (for example, bacterial reverse mutation assay; mammalian erythrocyte micronucleus test).
M4.9	870.2400	Primary Eye Irritation	NR	A primary eye irritation study is not required for the end-use product. If available, submit for review.
	870.1100 870.1300	Acute oral toxicity – Rat end-use product Acute inhalation toxicity – Rat end-use product	CR	An acute oral toxicity study on the end-use product is not required. If available, submit for review. An acute inhalation toxicity study is required for all fungal MPCAs that are formulated into dry end-use products.
		Other studies/data	CR	Required if other studies/data are available.
M5.0		Exposure assessment	CR	No testing required at this time. PMRA will conduct exposure assessment using relevant data/information from Parts M1.2, M2.7/885.1100 and toxicity/pathogenicity tests.
M7.0	885.2000	Food and feed residue studies	CR	The PMRA will determine whether residue studies are required based on M2.7.2 and M4.2.2. If toxins or other potentially toxic secondary metabolites have been demonstrated to be present in the end-use product, it must be demonstrated that toxicologically significant levels of these metabolites are absent in any edible food commodity otherwise an MRL may be required.

Data Code (Part)	USEPA OCSPP Guideline Number	Title	Data Required ¹	Additional Comments or Conditions ²
M8 ENVIRONMENTAL FATE / ENVIRONMENTAL EXPRESSION				
M8.1		Summary	CR	
M8.2 Laboratory studies				
M8.2.1	885.5200 885.5300 885.5400	Pure culture testing	CR	
M8.2.2	885.5300 885.5400	Microcosm testing	CR	
M8.3	885.5200	Greenhouse studies	CR	
M8.4	885.5200	Field studies	CR	
M8.5	885.5300	Expression in a freshwater environment (Other studies/data)	CR	
M9 Environmental toxicology/Nontarget organism testing³				
M9.1 Summary				
If sound scientific rationales are submitted in lieu of a study under DACO M9, the scientific rationale must be supported with results of a literature search on toxicity/pathogenicity of the MPCA, and/or closely related microorganisms, to support claims of low risk to the specific non-target group (for example, birds, fish). Environmental fate/expression data, as well as growth parameter (for example, maximum growth temperature) data may also be used to support a scientific rationale if claims of low risk are based on an expected low level of exposure to the MPCA under operational conditions of use (in other words, during and postapplication) of the end-use product or if MPCA cannot grow at host body temperatures.				
M9.2 Birds				
M9.2.1	885.4050	Avian oral	R	A 21-day avian oral study using the technical grade active ingredient as the test substance is required on one avian species, preferably bobwhite quail (<i>Colinus virginianus</i>) or mallard duck (<i>Anas platyrhynchos</i>).
M9.2.2	885.4100	Avian pulmonary, inhalation or injection	R	An intratracheal or intranasal instillation study at the MCC daily, for five successive days is required. If the technical grade active ingredient is suitable for aerosolization, an inhalation test at the MCC daily for five consecutive days may be conducted provided justification is provided. Alternatively, an IV or IP injection test, as a single dose of the MCC, may be substituted if certain criteria are met to simulate a maximum hazard challenge by bypassing the bird's primary defense mechanisms.
M9.3	885.4150	Wild mammals	CR	PMRA will conduct a review based on results of Part M4/885.000/870.000 series mammalian toxicity/pathogenicity studies to address potential concerns to wild mammals.

Data Code (Part)	USEPA OCSPP Guideline Number	Title	Data Required ¹	Additional Comments or Conditions ²
M9.4 Fish				
M9.4.1	885.4200	Freshwater fish	R	A 30-day static-renewal freshwater fish study using the technical grade active ingredient as the test substance is required on one cold water fish species, preferably rainbow trout (<i>Oncorhynchus mykiss</i>). Additional testing may be required on bluegill sunfish (<i>Lepomis macrochirus</i>) if there is potential for significant freshwater exposure.
M9.4.2	885.4280	Estuarine and marine fish/animal	CR	Conditionally required. Test data not required for a Tier I risk assessment of this MPCA.
M9.5 Arthropods				
M9.5.1	885.4340	Terrestrial arthropods Non-target insect testing	R	Toxicity/pathogenicity testing required using the technical grade active ingredient on at least 3 species of representative arthropods representing at least two of the following groups – parasitic dipterans, predaceous hemipterans, predaceous coleopterans, predaceous mites, predaceous neuropterans, parasitic hymenopterans. Exposure data (likelihood) or intended use pattern(s) may preclude non-target insect testing, and depend on adequate host range/identification of the MPCA.
M9.5.1	885.4380	Honey bee	R	Toxicity/pathogenicity testing using the technical grade active ingredient on honey bee is required. OCSPP Guideline 885.4380 recommends an exposure/observation period of 30 days. Since the OECD guidelines are used to test acute hazards/exposures to chemical pesticides, they are not appropriate for testing potential hazards of the MPCA to honeybees, as they do not include extended periods of duration.
M9.5.2	885.4240	Aquatic arthropods Freshwater invertebrate toxicity/pathogenicity testing	R	A 21-day life cycle study is required using the technical grade active ingredient on at least one or two (i.e., a benthic and/or planktonic) species such as a daphnid is required. Additional testing may be required if adverse effects are noted or if other species may be susceptible to the MPCA.
M9.6		Non-arthropod invertebrates	CR	Testing on nontarget non-arthropod invertebrates is only required if the MPCA is intended to control invertebrates such as annelids or mollusks.
M9.7		Microorganisms	CR	Information submitted on the mode of action in Part M2.7/OCSPP 885.1100 will be used to conduct a Tier I risk assessment on non-target microorganisms and microbially mediated processes.

Data Code (Part)	USEPA OCSPP Guideline Number	Title	Data Required ¹	Additional Comments or Conditions ²
M9.8 Plants				
M9.8.1	885.4300	Terrestrial non-target plant testing	R	For the technical grade active ingredient, toxicity/pathogenicity testing is required on those non-target plants expected to be contacted during operational conditions of use. Phytotoxicity/phytopathogenicity data from efficacy trials or from on non-crop or ornamental plants, and published literature may be useful in supporting a rationale.
M9.8.2		Aquatic plants (Tier 1)	R	For the technical grade active ingredient, toxicity/pathogenicity testing is required on non-target aquatic plants expected to be contacted during operational conditions of use. MPCAs that are taxonomically related to known plant pathogens may also require additional testing on potentially susceptible plant species. Knowledge of the mode of action may assist in determining the extent of testing needed for potential plant pathogens.
M9.9		Other studies/data	CR	Required if other studies/data are available.
SUMMARIES				
M12.5		Foreign reviews	CR	Individually submit the technical dossiers for the technical grade active ingredient and end-use product and all cited papers and studies.
M12.7	885.0001	Comprehensive data summaries	R	Required if other studies/data are available.

¹ R = Required; CR = Conditionally Required; NR = Not Required; NA = Not Applicable

² If a scientific rationale is submitted to address a data requirement, this rationale must be supported with sufficient information and/or data to address the underlying risk/hazard concerns. The scientific rationale must also be accompanied with all relevant published scientific literature and/or studies. The PMRA may require additional data and information during the preliminary review of this information.

³ For USEPA OCSPP 885 series testing (in other words, toxicity, pathogenicity testing) conducted by a laboratory contractor: Test substances (including sterile production filtrate, pesticidal active ingredient), other negative/positive controls used in the study and provided by the Sponsor must have a Certificate of Authenticity (in other words, Certificate of Analysis) included in the specific study report. A sufficient Certificate of Analysis (C of A) should indicate certain production information to the PMRA to facilitate review of the MPCA (technical grade active ingredient) under consideration for regulatory action. (1) name and strain; Lot or Batch Number; (2) 3 Dates: Manufacturing; Expiration; "Certified Analysis"; (3) Nature of test material: powder or liquid; amount provided to the test laboratory; storage requirements to maintain "certifiable limits" (4) Sponsor tested viability: Include final population count(s) of the Lot analyzed. A table of raw data and brief "SOP's or analytical procedures used for sampling and enumeration batch viability may be included in separate Appendices for the final study report. Separate C of A for test substance control(s): Inactive and/or Sterile Filtrate. The raw data should include the number of agar plates used for serial dilution plating (generally 3 dilution series) with at least 2 (preferably 4) "countable" plates/dilution to establish statistical confidence in techniques. The Certificate must be signed and dated by the Sponsor. Claims of potency should be substantiated by text and data showing how the potency was determined; if an SOP is available the SOP should be included in the Product Characterization/Analysis portion of the submission, OCSPP Guideline No. 885.1400), The Certificate of Analysis must be provided to the testing laboratory prior to initiation of the study. For a sterile production filtrate, provide the time of "harvest" (for example, 24 hours after initial start of fermentation, "logarithmic phase" or "lag phase", how the filtrate was prepared, size of filter, volume and diluent (if any). Indicate

the viability (cfu/ml) of the "harvested" batch. The sterile production filtrate should be prepared from the same Lot/Batch as the Test Substance, using aseptic technique and sterilized filter apparatus, and free of insoluble material and not cloudy or turbid, describe the color and contents of the filtrate (in other words, culture/fermentation medium), especially if it contains spent "fermentation media".

- 4 Technical Grade of the Active Ingredient is a material containing the MPCA in question which is produced commercially, or a manner equivalent to the planned commercial process, and to which no ingredient has been added intentionally except for purposes of MPCA growth or replication, or typical purification. The technical grade active ingredient is considered to be the purest preparation resulting from a typical production process, and is the preparation intended for distribution and/or formulation into a formulation intermediate or end-use product. Where techniques are used to alter the MPCA genetically, the genetically altered strain is considered as the basis for defining the technical grade of the MPCA. Each pesticidal product from genes that have been engineered into a microorganism also is considered as an active ingredient. The technical grade of each pesticidal product from introduced genes is that form which exists along with the technical grade of the MPCA in question after a typical production process.
- 5 Purest form of the microbial pest control agent (MPCA) is a preparation containing pesticidal functioning units of the MPCA in question obtained after the application of the most rigorous purification procedures. The purest form of the MPCA that can be obtained is a preparation that is free of any other biological forms and free of contaminating growth media or host substrate material.
- 6 End-use Product is the MPCA containing product that is intended for direct use or application for pest control purposes.

Appendix III 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.

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 6, Part 1.1 and Appendix V.
- (g) **Index:** of supporting data in the proper electronic and paper format. See Appendix IV.
- (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 *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 IV Directions for creating a data index

Create a data index using the [Electronic Pesticide Regulatory System \(e-PRS\)](#). The e-PRS web site provides step-by-step instructions for compiling and submitting documents and includes an e-Index Builder to ensure submissions are in the correct PRZ format.

Although numerous PMRA Guidelines and Guidance Documents give specific instructions as to how to submit documents to the PMRA, current guidance is that Applicants and Registrants now use e-PRS for document compilation and submission.

Appendix V Directions for creating a draft label for a microbial pest control product

Every registered microbial technical grade active ingredient, manufacturing concentrate and end-use product must include an associated registered label. Basic label requirements for microbial technical grade active ingredient, manufacturing concentrate and end-use product labels are outlined in the respective sections below. Certain statements are relevant only to technical grade active ingredient and manufacturing concentrate labels or end-use product labels, while other statements are required on all Classes of microbial products.

A Sample label for a microbial end-use product is included. For more detailed information on the preparation of technical grade active ingredient and product labels, refer to the [Changes to Label Requirements](#) page on the pesticides section of Canada.ca.

Principal display panel

1. Trade names and product type

- The trade name for the technical grade active ingredient, manufacturing concentrate or end-use product must match name on the application form.
- Or technical grade active ingredients and manufacturing concentrates, the name should include the term “Technical”; for example, *Beauveria bassiana* strain ABC Technical Powder. Otherwise, the term “Technical” or “Manufacturing Concentrate” must be indicated on the label.
- For end-use products, the product name should be specific to the product type and purpose. Otherwise, the Product Type (for example, Biological Insecticide, Biological Fungicide) must be indicated on the label.
- For technical grade active ingredient and manufacturing concentrate labels, a statement regarding the Product Type for which it is associated must be included on the label: for example, “For formulating/manufacturing of a biological insecticide/fungicide/insecticide”.
- The name should not be misleading or contain unacceptable or scientifically unsupportable adjectives, for example, natural, organic.

2. Physical form

- The physical form of the technical grade active ingredient, manufacturing concentrate or end-use product must be indicated; for example, liquid, solid, powder, emulsifiable suspension.
- For all microbial technical grade active ingredient, manufacturing concentrates and end-use products, the term “live organism” should also be indicated to identify that the contents are a biological product.

3. **Class designation**

- For technical grade active ingredient and manufacturing concentrate labels, the term “manufacturing” must be indicated on the label and must match the class designation indicated on application form. The terms “technical” or “manufacturing concentrate” are acceptable alternatives.
- For end-use products, the Class designation must be based on the intended use of the product and potential hazards and must match the class indicated on application form.
- Only one of the following class designation is accepted per product: “domestic”, “commercial”, “restricted”. For commercial products intended for agricultural or industrial or institutional uses, the terms “agricultural”, “industrial” or “institutional” are all considered acceptable provided that the term “commercial” is also included.

4. **Precautionary hazard symbols and signal words**

- Based on acute hazard testing, precautionary hazard symbols and signal words will be indicated on the principal display panel.
- The principal display panel of all microbial technical grade active ingredient, manufacturing concentrate and end-use products must state “potential sensitizer”.

5. **The principal display panel of all microbial labels must also include:**

- “Read the label before using” or, if the product labelling includes a brochure or pamphlet, “Read the label and attached brochure before using”.
- All microbial technical grade active ingredient and manufacturing concentrate labels must state “Prevent access by unauthorized personnel”.
- All “commercial” and “domestic” class end-use products must state: “Keep out of reach of children”.
- All “Restricted” class end-use products must state: “Prevent access by unauthorized personnel”.

6. **Active ingredient statement**

- For technical grade active ingredient, manufacturing concentrate and end-use product labels, the active ingredient guarantee must match that on the product specification form of the respective product and both of these are to reflect the concentration of the active ingredient(s) as described in Part M2.9.2 of these guidelines.

7. **Registration Number**

- A placeholder for the pending registration number must be included: “REGISTRATION NUMBER XXXXX PEST CONTROL PRODUCTS ACT”
- If size is a limiting factor, the statement may be revised as “REG. NO. XXXXX P.C.P. ACT.”

8. Net Contents

- Must be expressed in metric units; liquids are expressed in millilitres (mL) or litres (L) and solids or pressurized products are expressed in grams (g) or kilograms (kg).

9. Name and Full Postal Address of Registrant

- must match that in box 6 of the application form of the respective product
- the name, postal address and telephone number of a contact person in Canada or the US to which public inquiries may be directed



For DOMESTIC class products only that are of a very small size, points 6, 7, 8 and listed above, can appear on the secondary display panel.:

10. Date of manufacture

- The Date of Manufacture is required for all microbial technical grade active ingredient, manufacturing concentrate and end-use product labels (in order to determine end of storage period).
- A Lot Number and Expiry Date are optional.

Secondary display panel

1. DIRECTIONS FOR USE for technical grade active ingredient and manufacturing concentrate labels

- All technical grade active ingredient and manufacturing concentrate labels must include: "To be used only in the manufacture of a pesticide which is registered under the *Pest Control Product Act*." (The word "pesticide" can be replaced with biological insecticide, herbicide, fungicide, etc.)

2. DIRECTIONS FOR USE for end-use product labels

- For all microbial end-use product labels, the text must include complete information on application rates, how to apply the product, and use limitations.
- All microbial end-use product labels must state: "DO NOT contaminate irrigation or drinking water supplies or aquatic habitats by cleaning of equipment or disposal of wash water."
- A Restricted-Entry Interval (REI) will be specified on all microbial end-use product labels depending on the nature of the product and use pattern (for example, commercial, outdoor uses, greenhouse use, aquatic uses).
- Unless a specific preharvest interval (PHI) is being established, all microbial end-use product labels (with the exception of seed treatments and products applied preplant, pre-emergence or early postemergence) will include the standard PHI statement: "Preharvest interval: Product Name can be used up to and including the day of harvest. PHI = 0 days."

- Depending on the product class, the nature of the product and use pattern (for example, commercial, domestic, outdoor uses, greenhouse use, aerial applications, aquatic uses), standard label statements to limit environmental exposure will be included on microbial end-use product labels.
- All microbial end-use product labels must include a standard precautionary statement to limit exposure: "DO NOT contaminate irrigation or drinking water supplies or aquatic habitats by cleaning of equipment or disposal of wash water."

3. Human health 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 (technical grade active ingredient, manufacturing concentrate and end-use product labels), and the environment (end-use product labels only), along with instructions on how to alleviate such hazards.
- All technical grade active ingredient labels must include the following statements as standard precautionary measures to limit exposure: "Prevent access by unauthorized personnel. May cause sensitization. Avoid inhaling or breathing (spray mist or dust). Avoid contact with skin, eyes or clothing. Wash thoroughly with soap and water after handling. Remove contaminated clothing and wash before reuse."
- All Domestic class microbial end-use product labels must state: "Keep out of reach of children."
- All Commercial class microbial end-use product labels must state: "Keep out of reach of children and unauthorized personnel."
- All Restricted class microbial end-use product labels must state: "Prevent access by unauthorized personnel."
- All microbial end-use product labels must include the following precautionary statements to limit exposure for the user: "May cause sensitization." and in situations where the product is an eye irritant or where eye irritation studies have not been performed, the following statement "Avoid contact with skin, eyes and clothing. Avoid breathing or inhaling [spray mist or dust]." "Wash with soap and water after use." "Remove contaminated clothing and wash before reuse."
- For end-use product labels, additional product-specific precautionary health statements may be required based on hazard testing.
- Domestic class microbial end-use product do not require Personal Protective Equipment (PPE), unless hazard testing indicates otherwise.
- All Commercial and Restricted class microbial end-use product labels must include the following statement describing the standard PPE: "Wear a long-sleeved shirt, long pants, protective eyewear (goggles), waterproof gloves, socks and shoes and a NIOSH-approved particulate filtering facepiece respirator with any N, R or P filter when handling, mixing/loading or applying the product and during all clean-up/repair activities."

- Modifications to the standard PPE may be required based on hazard testing (for example, specific PPE for post-application workers).

4. **First aid and toxicological information**

- All microbial technical grade active ingredient, manufacturing concentrate and end-use product labels must include First Aid statements, as outlined in DIR2007-01, *First Aid Labelling Statements*.
- For microbial technical grade active ingredient and manufacturing concentrate labels, the standard toxicological information statement must be included: "TOXICOLOGICAL INFORMATION: Treat symptomatically."
- For all microbial end-use product labels, one of two standard toxicological information statement must also be included: for products that do not contain petroleum distillates, include: "TOXICOLOGICAL INFORMATION: Treat symptomatically." For products that contain >10% petroleum distillates, include: "TOXICOLOGICAL INFORMATION: This product contains a petroleum distillate solvent. Vomiting may cause aspiration pneumonia. Treat symptomatically."

5. **Environmental precautions (for microbial end-use product labels only)**

- For all microbial end-use products, specific environmental precaution statements may be required depending on the product classification, nature of the product, application method and hazards identified during testing.

6. **Storage statement**

- A storage statement must be included on all microbial technical grade active ingredient, manufacturing concentrate and end-use product labels to advise the user on appropriate storage conditions (for example, temperature range and light restrictions) and any other relevant information aimed at ensuring stability, performance and safety.
- For microbial technical grade active ingredient and manufacturing concentrate labels, the storage statement must state "[technical grade active ingredient name] should be used within [insert time period as supported by test data] from the date of manufacture when stored at [temperature as indicated by storage stability data]."
- For microbial end-use product labels, the storage statement must state "Store in original container, away from food or feed. [product name] should be used within [insert time period as supported by test data] from the date of manufacture when stored at [temperature as indicated by storage stability data]."

7. Disposal/decontamination

- As per DIR99-04, standard disposal statements will be included on the technical grade active ingredient, manufacturing concentrate, or end-use product label depending on the nature of the product, product class, type of container (for example, commercial/domestic; liquid/solid; non-recyclable/non-refillable/non-returnable or recyclable container) See DIR99-04, *Disposal Statements for Control Product Labels*.

8. Notice to user

- All microbial technical grade active ingredient and manufacturing concentrate labels (for example, Manufacturing class products) must include the statement: "NOTICE TO USER: This pest control product is to be used only in accordance with the directions on the label. It is an offence under the *Pest Control Products Act* to use this product in a way that is inconsistent with the directions on the label."
- All Commercial and Restricted product labels must state: "NOTICE TO USER: This pest control product is to be used only in accordance with the directions on the label. It is an offence under the *Pest Control Products Act* to use this product in a way that is inconsistent with the directions on the label."
- The NOTICE TO USER statement is not required for Domestic class products.

SAMPLE DRAFT LABEL

PRINCIPAL PANEL

BEETLE-B-GONE Flowable Biological Insecticide

LIQUID

LIVE ORGANISM

COMMERCIAL

READ THE LABEL BEFORE USING

KEEP OUT OF REACH OF CHILDREN

ACTIVE INGREDIENT: *Beauveria bassiana* strain ABC..... Minimum 1×10^9 conidia/mL

REGISTRATION NO: XXXXX PEST CONTROL PRODUCTS ACT

POTENTIAL SENSITIZER

[Precautionary symbols and signal words

belong here, as needed based on acute hazard testing]

Net Contents: 10 L
XYZ Biologicals Inc.
street name
City, Province
Postal Code

Tel Number: 1-800-666-1212

Date of Manufacture:

Secondary panel

General information: BEETLE-B-GONE contains live spores of the fungus, *Beauveria bassiana* strain ABC. BEETLE-B-GONE is a mycoinsecticide formulated to control beetles in canola.

Directions for use: Shake well before use. Mix BEETLE-B-GONE at a rate of 0.5–1 L per 400 Liters spray volume. To mix, fill spray tank with half the desired amount of water and start agitation. Shake BEETLE-B-GONE to suspend spores then slowly add the appropriate quantity to obtain the required concentration to spray tank. Add remainder of desired amount of water. Do not mix more BEETLE-B-GONE than needed for that day. Do not mix BEETLE-B-GONE the day before application. Spores will die if left overnight or longer in the spray tank.

Application frequency: Begin treatment of crops at the first appearance of the beetles. Typically, it takes 7-10 days after the first spray to see control. BEETLE-B-GONE is most effective when used early, before high beetle populations develop. Reapply as necessary under a pest management program that includes close scouting.

Apply BEETLE-B-GONE at 5-10 day intervals. Repeat applications for as long as pest pressure persists. There is no limit on the number of applications or total amount of BEETLE-B-GONE which can be applied in one season. DO NOT tank mix BEETLE-B-GONE with any fungicide.

Application rate: 0.5-1L per 400 Liters spray volume. Apply at 83 mL/ha in 400 L of water per hectare by ground sprayer.

As this product is not registered for the control of pests in aquatic systems, DO NOT use to control aquatic pests.

DO NOT contaminate irrigation or drinking water supplies or aquatic habitats by cleaning of equipment or disposal of wash water.

DO NOT apply using aerial application equipment.

Restricted-Entry Interval (REI): DO NOT allow worker entry into treated areas for 4 hours or until sprays have dried unless wearing waterproof gloves, long-sleeved shirt, long pants, socks and shoes.

Preharvest interval: BEETLE-B-GONE can be used up to and including the day of harvest. PHI = 0 days.

Precautions: KEEP OUT OF REACH OF CHILDREN. May cause sensitization. Avoid contact with skin, eyes and clothing. Avoid breathing spray mist. Wear a long-sleeved shirt, long pants, protective eyewear (goggles), waterproof gloves, socks and shoes and a NIOSH-approved particulate filtering facepiece respirator with any N, R or P filter when handling, mixing/loading or applying the product and during all clean-up/repair activities. Wash

thoroughly with soap and water after handling. Remove contaminated clothing and wash before reuse. Apply only to agricultural crops when the potential for drift to areas of human habitation and human activity, such as houses, cottages, schools and recreational areas is minimal. Take into consideration wind speed, wind direction, temperature inversions, application equipment and sprayer settings.

First aid:

IF SWALLOWED: Call a poison control centre or doctor immediately for treatment advice. Have person sip a glass of water if able to swallow. Do not induce vomiting unless told to do so by a poison control centre or doctor. Do not give anything by mouth to an unconscious person.

IF ON SKIN OR CLOTHING: Take off contaminated clothing. Rinse skin immediately with plenty of water for 15–20 minutes. Call a poison control centre or doctor for treatment advice.

IF INHALED: Move person to fresh air. If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably by mouth-to-mouth, if possible. Call a poison control centre or doctor for further treatment advice.

IF IN EYES: Hold eye open and rinse slowly and gently with water for 15–20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. Call a poison control centre or doctor for treatment advice.

GENERAL: Seek medical attention immediately if irritation or signs of toxicity occur and persist or is severe. Take container, label or product name and Pest Control Product Registration Number with you when seeking medical attention.

Toxicological information: Treat symptomatically.

Environmental precautions: To reduce runoff from treated areas into aquatic habitats, avoid application to areas with a moderate to steep slope, compacted soil or clay. Avoid application when heavy rain is forecast. Contamination of aquatic areas as a result of runoff may be reduced by including a vegetative filter strip between the treated area and the edge of the water body.

Storage: BEETLE-B-GONE should be used within 9 months from the date of manufacture when stored at 25°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. Store away from food and feed.

Disposal/Decontamination:

1. Triple or pressure-rinse the empty container. Add the rinsings 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, contact the manufacturer or the provincial regulatory agency. Contact the manufacturer and the provincial regulatory agency in case of spill, and for clean-up of spills.

Notice to user: This pest control product is to be used only in accordance with the directions on the label. It is an offence under the *Pest Control Products Act* to use this product in a way that is inconsistent with the directions on the label.

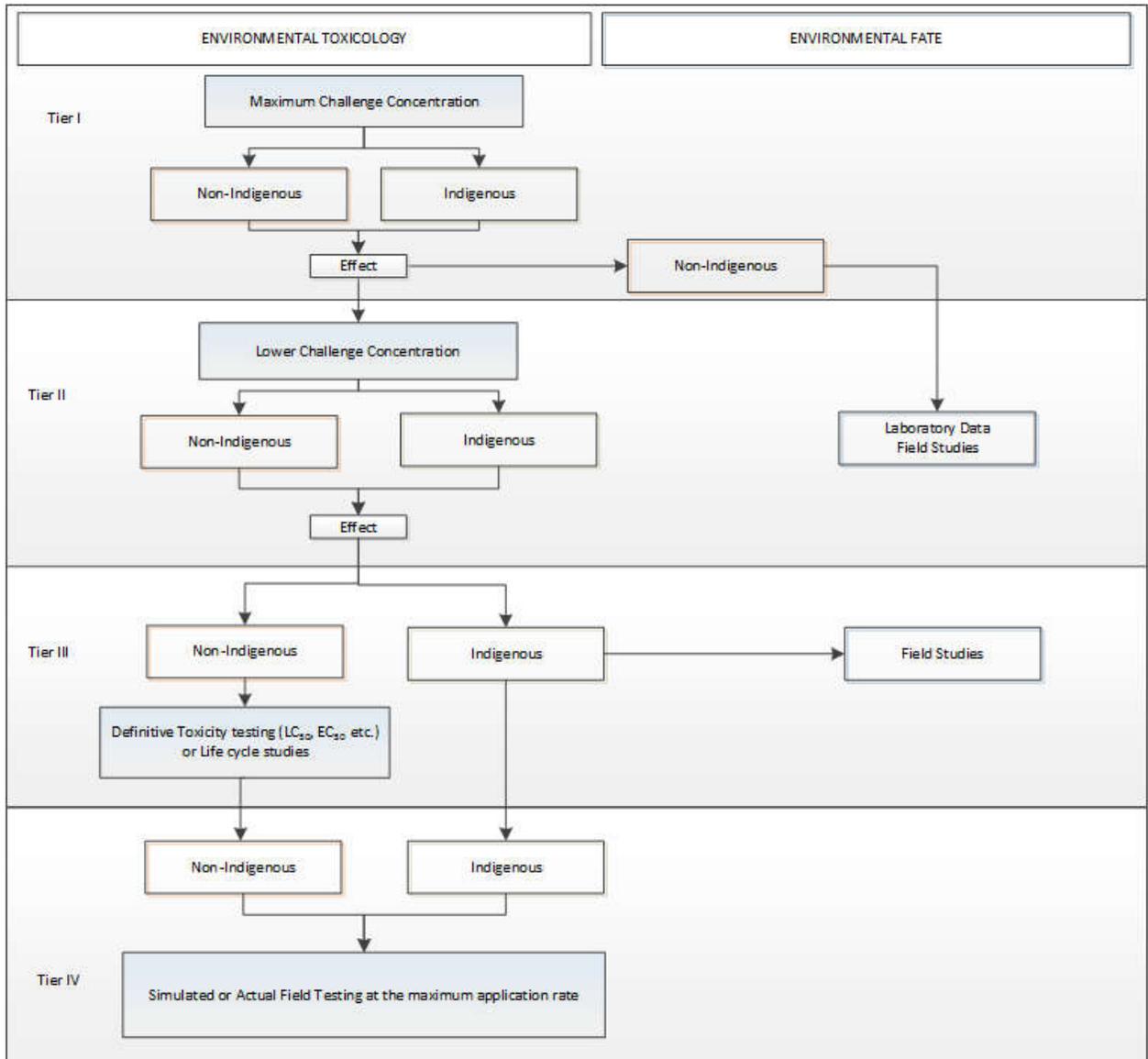
Appendix VI Human health and safety testing requirements

Test Animal Species	DACO	Data Required	Test Substance
Part M4 Human Health and Safety Testing			
Summary	M4.1	R	n/a
Infectivity and toxicity	M4.2		
Summary	M4.2.1	R	n/a
Acute oral: Rat (preferred)	M4.2.2	R	technical grade active ingredient
Acute pulmonary: Rat (preferred)	M4.2.3	R	technical grade active ingredient
Acute IV or IP infectivity	M4.3		
Summary	M4.3.1	R	n/a
Intravenous infectivity (bacteria or viruses): Newly weaned mouse or hamster	M4.3.2	R	MPCA
Intraperitoneal infectivity (fungi or protozoa): Rat or mouse	M4.3.3	R	MPCA
Acute dermal toxicity: Rabbit	M4.4	R	end-use product
Irritation	M4.5		
Summary	M4.5.1	R	n/a
Dermal irritation study: Rabbit	M4.5.2	R	end-use product
Reporting of hypersensitivity incidence	M4.6	R	MPCA or end-use product
Tissue culture (viral agents only)	M4.7	R	MPCA
Genotoxic potential (fungi or actinomycetes)	M4.8	CR	MPCA
Acute inhalation toxicity	M4.9	CR	end-use product

Appendix VII Microbial pesticide ecozones of Canada



Appendix VIII Environmental toxicology and environmental fate testing tiers



Appendix IX Environmental toxicology and fate testing requirements based on use pattern

Test	DACO	Use Pattern				Test Substance	Type of Test
		Terrestrial	Aquatic	Forestry and Domestic Outdoor	Greenhouse*		
Tier I							
Avian Oral	M9.2.1	R	R	R	CR	technical grade active ingredient or end-use product	MCC
Avian Pulmonary, Inhalation or Injection	M9.2.2	R	R	R	CR	technical grade active ingredient or end-use product	MCC
Wild Mammals	M9.3	CR	CR	CR	CR	technical grade active ingredient or end-use product	MCC
Fish: Freshwater	M9.4.1	R	R	R	CR	technical grade active ingredient or end-use product	MCC
Fish: Estuarine or Marine	M9.4.2	CR	CR	CR	CR	technical grade active ingredient or end-use product	MCC
Arthropods: Terrestrial	M9.5.1	R	R	R	CR	technical grade active ingredient or end-use product	MCC
Arthropods: Aquatic	M9.5.2	R	R	R	CR	technical grade active ingredient or end-use product	MCC
Non-Arthropod Invertebrates: Terrestrial	M9.6.1	R	R	R	CR	technical grade active ingredient or end-use product	MCC
Non-Arthropod Invertebrates: Aquatic	M9.6.2	R	R	R	CR	technical grade active ingredient or end-use product	MCC
Microorganisms	M9.7	CR	CR	CR	CR	technical grade active ingredient or end-use product	MCC

Test	DACO	Use Pattern				Test Substance	Type of Test
		Terrestrial	Aquatic	Forestry and Domestic Outdoor	Greenhouse*		
Plants: Terrestrial	M9.8.1	R	R	R	CR	technical grade active ingredient or end-use product	MCC
Plants: Aquatic	M9.8.2	R	R	R	CR	technical grade active ingredient or end-use product	MCC
Tier II							
Avian Oral	M9.2.1	CR	CR	CR	CR	technical grade active ingredient or end-use product	LCC
Avian Pulmonary, Inhalation or Injection	M9.2.2	CR	CR	CR	CR	technical grade active ingredient or end-use product	LCC
Wild Mammals	M9.3	CR	CR	CR	CR	technical grade active ingredient or end-use product	LCC
Fish: Freshwater	M9.4.1	CR	CR	CR	CR	technical grade active ingredient or end-use product	LCC
Fish: Estuarine or Marine	M9.4.2	CR	CR	CR	CR	technical grade active ingredient or end-use product	LCC
Arthropods: Terrestrial	M9.5.1	CR	CR	CR	CR	technical grade active ingredient or end-use product	LCC
Arthropods: Aquatic	M9.5.2	CR	CR	CR	CR	technical grade active ingredient or end-use product	LCC
Non-Arthropod Invertebrates: Terrestrial	M9.6.1	CR	CR	CR	CR	technical grade active ingredient or end-use product	LCC
Non-Arthropod Invertebrates: Aquatic	M9.6.2	CR	CR	CR	CR	technical grade active ingredient or end-use product	LCC
Microorganisms	M9.7	CR	CR	CR	CR	technical grade active ingredient or end-use product	LCC

Test	DACO	Use Pattern				Test Substance	Type of Test
		Terrestrial	Aquatic	Forestry and Domestic Outdoor	Greenhouse*		
Plants: Terrestrial	M9.8.1	CR	CR	CR	CR	technical grade active ingredient or end-use product	LCC
Plants: Aquatic	M9.8.2	CR	CR	CR	CR	technical grade active ingredient or end-use product	LCC
Environmental Fate: Pure Culture Testing	M8.2.1	CR	CR	CR	CR	MPCA	NA
Environmental Fate: Microcosm Testing	M8.2.2	CR	CR	CR	CR	technical grade active ingredient or end-use product	MCC
Environmental Fate: Greenhouse Studies	M8.3	CR	CR	CR	CR	end-use product	MAR
Environmental Fate: Small or Large Scale Field Studies	M8.4	CR	CR	CR	CR	end-use product	MAR
Tier III							
Definitive Toxicity Testing of Non-Target Organisms	M9.2.1–M9.8.2	CR	CR	CR	CR	technical grade active ingredient or end-use product	Multiple Concentrations
Life Cycle Testing of Non-Target Organisms	M9.2.1–M9.8.2	CR	CR	CR	CR	technical grade active ingredient or end-use product	EEC
Environmental Fate: Small- or Large-Scale Field Studies	M8.2.4	CR	CR	CR	CR	end-use product	MAR
Tier IV							
Environmental Toxicology: Small-Scale Field Studies	M9.2.1–M9.8.2	CR	CR	CR	CR	end-use product	MAR

* For greenhouse uses, the need for environmental testing will depend largely on the type (in other words, the design and operation of greenhouse facilities in which the end-use product will be applied) and the degree of environmental exposure (in other words, the level of containment) anticipated under operational conditions of use. A pre-submission consultation is recommended for products proposed for greenhouse uses to identify specific data requirements for the MPCA in question.

Appendix X Summary of non-target toxicology testing

Tier	Type of Test	Form of Microbial Agent	Non-target to be Tested
I	Maximum Challenge Concentration	technical grade active ingredient or end-use product	Taxonomically related Infected by MPCA High exposure potential Similar physiology Susceptible to related pathogens Representative species from 7 broad taxonomic groups
II	Lower Challenge Concentration	technical grade active ingredient or end-use product	Adversely affected species from Tier I toxicology tests
III*	Definitive Studies (Determination of an LC ₅₀ , LD ₅₀ , EC ₅₀) and Life Cycle Tests	technical grade active ingredient or end-use product	Adversely affected species from Tier II toxicology tests
IV	Simulated or Actual Field Testing such as Small-Scale Field Studies (Environmental toxicology)	end-use product	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 (DACO M9.5.1)

Group	Freshwater	Estuarine or Marine	Terrestrial
Arachnida	Araneae		Araneae Scorpionida
Acari (mites and ticks)			Eriophyidae Phytoseiidae Stigmaeidae Tetranychidae Tydeidae
Crustacea	Cladocera ¹ Copepoda Decapoda Amphipoda	Anostraca Copepoda Cirripedia Mysidacea Amphipoda Decapoda	Isopoda
Insecta	Ephemeroptera Odonata Plecoptera Megaloptera Trichoptera Lepidoptera Coleoptera Diptera Hymenoptera		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 (DACO M9.8)

Terrestrial	Aquatic
Apiaceae (Umbelliferae)	Lemnaceae
Asteraceae (Compositae)	Potamogetonaceae
Brassicaceae (Cruciferae)	Haloragaceae
Chenopodiaceae	Typhaceae
Cucurbitaceae	Cyperaceae
Fabaceae (Leguminosae)	Alismaceae
	Chlorophyceae (green algae) ²
	Cyanophyceae (blue-green) ²
	Bacillariophyceae (diatom) ²
Liliaceae	
Malvaceae	
Poaceae (Gramineae)	
Polygonaceae	
Rosaceae	
Solanaceae	
Pinaceae ¹	

¹ Required for microbial products with forestry uses.

² For microbial products with aquatic use patterns and for terrestrial uses where aquatic exposure is anticipated, at least one representative species from each of the algal families must also be tested.

Appendix XIII List of relevant publications

Regulatory Authority

Pest Control Products Act and Regulations

Food and Drugs Act and Regulations

PMRA Companion Guidance Documents

Research Permit Guidelines for Microbial Pest Control Products

Guidelines for Efficacy Assessment of Chemical Pesticides

Guidelines for Efficacy Assessment of Herbicides and Plant Growth Regulators

Assessment of the Economic Benefits of Pesticides

Guidelines for Efficacy Assessment of Fungicides, Bactericides and Nematocides

Management of Submissions Policy, Pest Management Regulatory Agency

Good Laboratory Practice

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

OECD (2014), *OECD Issue Paper on Microbial Contaminants Limits for Microbial Pest Control Products*, Series on Pesticides and Biocides, No. 65, OECD Publishing, Paris, <https://doi.org/10.1787/9789264221642-en>.

OECD 2018a. Working Document on the Risk Assessment of Secondary Metabolites of Microbial Biocontrol Agents Series on Pesticides No. 98. 21 November 2018. [http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mon o\(2018\)33&doclanguage=en](http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mon o(2018)33&doclanguage=en)