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OECD GUIDANCE TO THE ENVIRONMENTAL SAFETY EVALUATION OF MICROBIAL BIOCONTROL AGENTS

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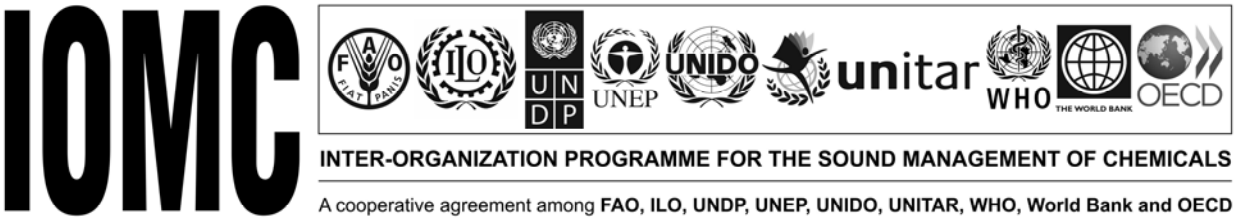
OECD Guidance to the Environmental Safety Evaluation of Microbial Biocontrol Agents



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FOREWORD

This document dealing with biological pesticides is intended to provide guidance to both industry and regulatory authorities, in the context of applications for the approval of microbial biological control agents (mBCAs), and for the registration of microbial biological control products (mBCPs). This document has been developed in the framework of the OECD BioPesticides Steering Group (BPSG), a sub-group of the OECD Working Group on Pesticides (WGP), that helps member countries harmonise the methods and approaches used to assess biological pesticides and to improve the efficiency of control procedures.

The BPSG regards its work as "dynamic" intended to address scientific issues as they arise and which may be impediments to harmonisation and work-sharing of microbial dossiers and monographs. Consequently, the BPSG has endeavoured to address and develop guidance on other issues as needed. The present document represents one such area, namely guidance on the environmental safety evaluation of microbial biopesticides.

The Netherlands and Germany served together as lead countries in the preparation of this guidance document. It was developed with the aim of harmonizing risk assessment of mBCAs. In order to achieve that objective **a risk assessment decision scheme** was developed which clarifies all the individual steps to be made in the risk assessment. The various sections of this guidance describe each individual step of that scheme in detail, with the knowledge currently available.

The use of mBCAs in this guidance is restricted to crop protection for outdoor applications. Main groups of mBCAs are bacteria, fungi, viruses, protozoa and microsporidia. The final goal of applying this decision scheme is to discern whether in view of the intended use of the product, the submitted data, information and tests, the potential risk to the environment is considered acceptable or not.

This OECD guidance document was prepared in consultation with OECD member countries and the regulated industry participating in the OECD BPSG. It is consistent with the OECD guidelines and criteria for the evaluation of dossiers and for the preparation of reports by regulatory authorities (OECD Monograph and Dossier Guidance for Microbials, published in 2004, and later revised in 2006).

The present guidance document received final approval of the OECD BPSG by written procedure ending on 28 June 2011 and of the OECD WGP by written procedure ending 17 November 2011.

This document is being published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology, which has agreed that it be unclassified and made available to the public.

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Glossary and Abbreviations

Accumulation	The rate of decline of viable CFUs is lower than possible increases through reproduction and/or repeated use of the mBCA
BPPD	Biopesticides & Pollution Prevention Division (Office of Pesticide Programs, US Environmental Protection Agency)
BPSG	Biopesticides Steering Group
Bt	*Bacillus thuringiensis*
Btk	*Bacillus thuringiensis* subsp. *kurstaki*
CCA	Chemical Control Agent
DAR	Draft Assessment Report: Monographs prepared by a rapporteur member state in the context of inclusion of active substances in Annex I of the Council Directive 91/414/EEC
DNA	Deoxyribonucleic acid
CFU	Colony Forming Unit (synonym: microbial unit)
EC	European Commission
EC_{50}	Median Effective Concentration
EFSA	European Food Safety Authority
EPF	Entomopathogenic Fungi
EPPO	European and Mediterranean Plant Protection Organization
ER_{50}	Median Effective Rate
EU	European Union
GAP	Good Agricultural Practice
GV	Granulovirus
HQ	Hazard Quotient
IOBC	International Organisation for Biological Control (of Noxious Animals and Plants)
Infectivity	The ability of a microorganism to cross or evade natural host barriers to infection (EPA, 1996)
IPM	Integrated Pest Management
LC_{50}	Median Lethal Concentration
LR_{50}	Median Lethal Rate
mBCA	Microbial Biological Control Agent
MCC	Maximum Challenge Concentration
MDD	Maximum Daily Dose
MHD	Maximum Hazard Dose
MHC	Maximum Hazard Concentration
MoS	Margin of Safety
MPCA	Microbial Pesticide Control Agent (for the sake of consistency the term "MPCA" is replaced by "mBCA" in the present document)
Multiplication	The regeneration of the microorganism
NOEC	No Observed Effect Concentration
NOEL	No Observed Effect Level
NPV	Nucleopolyhedrovirus
NTA	Non-Target Arthropod

NTO	Non-Target Organism
OECD	Organisation for Economic Co-operation and Development
OCSPP	Office of Chemical Safety and Pollution Prevention
OPPTS	Office of Prevention, Pesticides and Toxic Substances. On April 22, 2010 this name was changed to "Office of Chemical Safety and Pollution Prevention" (OCSPP)
Pathogenicity	The ability to inflict injury and damage in the host after infection, and depends on host resistance or susceptibility (EPA, 1996)
Persistence	Survival and/or establishment for longer periods.
PEC	Predicted Environmental Concentration
PIEC	Predicted Initial Environmental Concentration
PMRA	Pest Management Regulatory Agency. Government department in Canada. Environment Canada is another Government department
PPP	Plant Protection Product
PRAPeR	Pesticide Risk Assessment Peer Review (EFSAs PRAPeR Unit is responsible for the risk assessment in the EU peer review programme of active substances)
REBECA	Regulation of Biological Control Agents
STP	Sewage water Treatment Plant
TER	Toxicity/Exposure Ratio
TGAI	Technical Grade of Active Ingredient
Toxicity	The injury or damage in a host caused by a poison or toxin where infection by and/or replication or viability of the microorganism are not necessarily required (EPA, 1996).
US EPA	United States Environmental Protection Agency
UV	Ultraviolet
wt	weight

Introduction

This guidance to the environmental safety evaluation of microbial biocontrol agents (mBCAs) is the follow-up of the risk assessment scheme developed by Mensink (2005). This risk assessment scheme was later published by Mensink and Scheepmaker (2007). In the OECD meeting in Arlington (April 2008) it was decided that this risk assessment scheme by Mensink (2005) should be adapted into an OECD guidance document so as to provide an additional tool to risk assessors.

The authors[1] of this current OECD guidance are foremost familiar with the EU regulations but an attempt has been made to generalize the guidance without referring to a particular regulation. The scheme used by Mensink (2005) was restructured, following the natural, most logical flow of a risk assessment. Thus, a harmonised decision scheme is anticipated that can be used by risk assessors of all nationalities.

The use of mBCAs in this guidance is restricted to crop protection. Main groups of mBCAs are bacteria, fungi, viruses, protozoa and microsporidia.

The final goal is to discern whether in view of the intended use of the product and the submitted data, information and tests, the potential risk to the environment is considered acceptable or not. The risk assessment scheme and its guidance comprise the basic information and risk assessment items on which consensus was shown by the members of the BPSG. This guidance forms the platform for further data processing and integration. The risk assessment scheme is not intended for the use of genetically modified mBCAs as, at least in Europe, these are assessed under another legislation (Directive 2001/18/EC). This mBCA guidance can serve as input for the risk assessment for genetically modified mBCAs.

The risk assessment scheme

The environmental risk assessment terminates in “Risk acceptable” or “Risk not acceptable”.

Should any path in the risk assessment scheme lead to “Risk not acceptable”, the regulatory authority will consider all available information in order to determine whether registration may still be desirable under certain conditions (e.g., proposed mBCA will replace a toxic pesticide). The regulatory authority will only reject proposed uses in the last instance.

It should also be noted that this scheme does not directly lead to a final authorization as input from other risk assessment areas such as the human risk assessment also needs to be considered.

---

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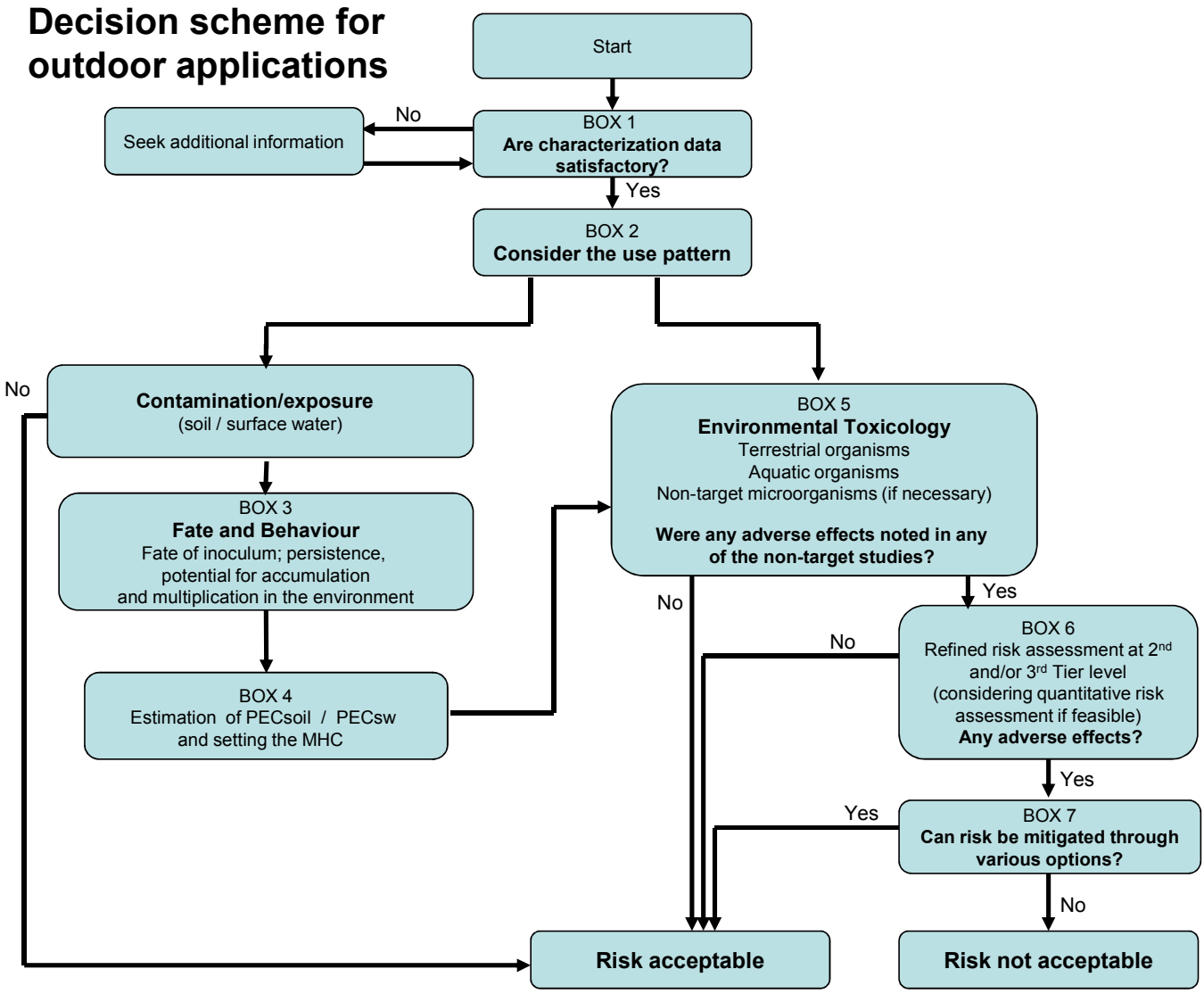
Decision scheme for outdoor applications
Start
BOX 1
Are characterization data satisfactory?
No
Seek additional information
Yes
BOX 2
Consider the use pattern
No
Contamination/exposure
(soil / surface water)
BOX 3
Fate and Behaviour
Fate of inoculum; persistence, potential for accumulation and multiplication in the environment
BOX 4
Estimation of PECsoil / PECsw and setting the MHC
BOX 5
Environmental Toxicology
Terrestrial organisms
Aquatic organisms
Non-target microorganisms (if necessary)
Were any adverse effects noted in any of the non-target studies?
No
Yes
BOX 6
Refined risk assessment at 2nd and/or 3rd Tier level (considering quantitative risk assessment if feasible)
Any adverse effects?
No
Yes
BOX 7
Can risk be mitigated through various options?
Yes
No
Risk acceptable
Risk not acceptable

1. Characterisation of the mBCA (BOX 1 of decision scheme)

Basic knowledge of the specific microorganism is required before starting the risk evaluation. Special attention should be given to non-indigenous and those indigenous mBCAs that are applied in different ecological compartments than they naturally occur (*e.g.* soil organisms applied to blossoms). These require a case-by-case approach at the strain level. Indigenous isolates though should still require close scrutiny since these were selected for a specific biological property or function that may not be available in other isolates. In some cases, the mBCA could, in theory, differ in some aspects from the species.

The following basic characteristics of the microorganisms should be considered

1. taxonomy;
2. the biology of the microorganism;
    - origin;
    - mode of action;
    - host range;
    - ability to survive in various environments (fate);
    - niche, natural occurrence;
    - life cycle including reproduction methods and dispersal mechanisms.
3. methods to identify the mBCA (*e.g.* molecular techniques, morphology, growth substrates/conditions)

1.1 Possible modes of action

The effect of a mBCA depends on the mode(s) of action of the microorganism. The effect can be the combination of several modes of action as given below, with different processes occurring in parallel. Not all modes of action for each species of microorganism may already be discovered.

Possible modes of action:

1. antibiosis (*e.g.* production of toxins, fungal bioactives (metabolites), production of cell-wall degrading enzymes);
2. toxicity (note: antibiosis is a wider term including the action of toxins);
3. pathogenicity (note: antibiosis and pathogenicity might be overlapping terms. Usually pathogenicity manifests in effects like mortality or obvious sublethal effects. Dose effect relation may however be unclear);
4. induction of plant resistance;

5. interference with the virulence of a pathogenic target organism;

6. endophytic growth;

7. root colonisation;

8. competition for ecological niche (*e.g.* nutrients, habitats);

9. parasitisation.

Background information on fungal bioactives (metabolites):

According to EU legislation, relevant metabolites need to be identified and further dealt with in a separate dossier. So far, this has not occurred yet.

Regulation (EU) No 544/2011 (EU, 2011) states that, if the product action is known to be due to the residual effect of a toxin/metabolite or if significant residues of toxins/metabolites are to be expected not related to the effect of the active substance, a dossier for the toxin/metabolite has to be submitted in accordance with the requirements of Annexes IIA and, where specified, the relevant parts of Annex IIIA.

Regulation (EU) No 544/2011 (EU, 2011) further states under the section fate and behaviour in the environment: Any relevant metabolites (*i.e.* of concern for human health and/or the environment) formed by the test organism under any relevant environmental conditions should be characterised. If relevant metabolites are present in or produced by the micro-organism, data as outlined under Annex II, Part A, point 7 may be required, if all of the following conditions are met:

- the relevant metabolite is stable outside the microorganism, see point 2.8, and
- a toxic effect of the relevant metabolite is independent of the presence of the micro-organism, and
- the relevant metabolite is expected to occur in the environment in concentrations considerably higher than under natural conditions.

Bacteria and fungi may secrete a wide range of metabolites, mostly products of secondary metabolism. These metabolites, including toxins, serve different functions depending on the ecological niche of the microbe, and may occur in many environmental compartments (in particular in soil, surface waters, groundwater and air), in animal feed or in food for human consumers. These substances could vary in structure, some are simple organic molecules such as antimicrobial agents produced by fungi and others are peptides or proteins.

A complete identification and characterisation of all metabolites which are produced by bacteria and fungi under different (environmental) conditions will not be feasible for technical reasons. However, the potential for the microorganism to produce metabolites that could be harmful to humans and/or the environment should be assessed, using information on the mode of action, the potential of related species and strains to produce relevant metabolites/toxins, adverse effects observed in the (eco)toxicity tests, and all other relevant information in published scientific literature.

The information provided must be sufficient to permit the performance of a risk assessment for man and/or environment, arising from potential exposure to the microorganism and metabolites (toxins).

1.2 Host range

The basic information on the host range should already give some indication on the possibility of infectivity or pathogenicity to other species than the target organism. For example, baculoviruses have narrow host ranges usually confined to one or a few species of closely related insects.

However, it is noteworthy to distinguish between physiological and ecological susceptibility. In general, it is difficult to compare between physiological host range (determined under laboratory conditions) and ecological host range (determined under field conditions). In a literature review by Roy and Cottrell (2008), it was concluded that many factors affecting pathogenicity under both laboratory and field conditions must be taken into account to make sense of how physiological susceptibility relates, if at all, to ecological susceptibility. Furthermore, it could be concluded that the lack of physiological susceptibility should be a reliable indicator that a specific strain or isolate of a pathogen will be highly unlikely to be infective under field conditions. Studies examining pathogen host range generally show that physiological susceptibility greatly exaggerates ecological susceptibility (Hajek *et al.*, 1995, 1996; Solter and Maddox, 1998). In general, information on the host range should be provided in an early stage of assessment based on a transparently conducted literature search using widely accepted databases. If literature searches do not yield sufficient results for a new isolate, studies (experimental data) on the host range should be provided.

Host ranges of selected groups of mBCAs consisting of entomopathogenic fungi, entomopathogenic bacteria and baculoviruses

The examples given below are mainly based on the experience of the assessment of 4th list substances under Commission Regulation (EC) No 2229/2004 (EU, 2004). The examples are therefore not exhaustive. At first glance, differences in the extent of (physiological) host ranges between EPF, bacteria und viruses are obvious.

Entomopathogenic fungi (EPF)

The host ranges of the entomopathogenic fungi *Beauveria bassiana* (different strains) and *Metarhizium anisopliae* surpass the borders of subphylum. *Beauveria bassiana* is able to attack arthropods of the subphylum Hexopoda (white flies, thrips, aphids), the subphylum Chelicerata (mites), Crustacea (sowbugs) and Myriapoda (millipedes). The host range of *M. anisopliae* includes Coleoptera belonging to the subphylum Hexapoda as well as mites belonging to the subphylum of Chelicerata.

Entomopathogenic bacteria

Entomopathogenic sporeforming bacteria such as *Bacillus thuringiensis* (subsp. *kurstaki, aizawai, tenebrionis* and *israelensis)* have specific modes of action due to the presence of δ-endotoxins from the Cry-protein family and other factors that selectively destroy the gut of target insects. Depending on the pathotype or combination of bioinsecticidal Cry-proteins this leads to different preferential activity against target pest species within the insect orders Lepidoptera, Coleoptera and Diptera. The range of susceptible species also covers non-target insects. For instance *Bt* subsp. *kurstaki* is primarily active against Lepidoptera, but has also been recorded as active against other insect orders such as Coleoptera, Diptera, Hymenoptera, Hemiptera, Isoptera, Phthiraptera, Siphonaptera, Thysanoptera, Neuroptera, Ephemeroptera (Glare and O'Callaghan, 2000). In contrast to the example on EPF above, affected orders mentioned above are within the same subphylum.

It should be noted that also nematicidal activity has been reported in the open literature for several *Bt* isolates (Leyns *et al.*, 1995). Besides the ability to secrete thermostable nucleotide β-exotoxins, some *Bt* strains are able to produce thermolabile factors with nematicidal activity (Mozgovaya *et al.*, 2002). Due to the nonspecific toxicity of β-exotoxins to insects and mammalian cells, β-exotoxins containing *Bt* products have been banned from public use and shall be free from β-exotoxins when tested with fly larvae toxicity tests or an equivalent HPLC method (WHO, 1999). In this context, an improved fly bioassay was recently developed by Mac Innes and Bouwer (2009) that is suitable for the routine screening of *Bt* strains for β-exotoxins.

For the diverse group of bacterial mBCAs other than *Bt* broad host specificities cannot be excluded due to their variety and numerous potential modes of action. Nevertheless, bacterial entomopathogens other than *Bt* have been developed which seem to indicate a higher degree of host specificity compared to commercially available *Bt*-preparations. For example, the activity of the sporeforming bacterium *Bacillus sphaericus* is restricted to certain dipteran species (Glare and O'Callaghan, 2000). Another example is the non-sporeforming bacterium *Serratia entomophila* with activity against only a limited range of scarab species (Jackson *et al.*, 1991).

Baculoviruses

Baculoviruses belong to a family of rod-shaped, enveloped viruses with a circular double stranded DNA and are divided into the genera granuloviruses (GV) and nucleopolyhedroviruses (NPV) on the basis of occlusion body morphology (OECD, 2002). Because of their specific mode of action baculoviruses have a very narrow host range and are strictly host-specific to certain arthropod species. In view of the host specificity, a distinction can be made between the genera GV and NPV.

The host range of NPV is usually restricted to one or a few species of the genus or family. However, there are NPV that exhibit a larger host range such as *Autographa californica* NPV infecting more than 30 species from about 10 insect families (OECD, 2002). In contrast to NPV, the host range of GV appears to be even narrower and mostly restricted to very few species of a single family (e.g. the family Tortricidae for *Cydia pomonella* GV). An increasing body of evidence suggests that Baculoviruses such as *Cydia pomonella* GV represent the most specific pesticidal agent of all microbials and chemicals (Hauschild, 2011).

Given the varying host specificities among different groups of mBCAs, it should be noted that, although the risk caused by entomopathogenic fungi seems to be higher than by entomopathogenic bacteria or viruses, sustainable adverse effects to arthropods under field conditions are hardly observable due to specific environmental conditions (*e.g.* microclimate, high humidity) needed for germination and attacking the host. In general it can be concluded, that risk assessments based on laboratory determined host ranges often overestimate the risk compared to field conditions. Therefore a distinction between the physiological and the ecological host range has to be made.

1.3 The selection of appropriate test species

The choice of non-target species for testing as well as the specific test methods (pathogenicity tests vs. standard toxicity tests) should be related to the mode of action and the proposed use of the microorganism in the field.

Australia suggests the approach of radial taxonomic testing in risk assessment procedures for mBCAs. Radial taxonomic testing essentially involves a taxonomic analysis expanding out from the target species to look at possible effects on related species, genera, families, tribes, orders etc. (for more information

please refer to the study by Weidemann and Tebeest, 1990). The same approach is taken by Environment Canada and is termed "centrifugal taxonomic approach" (PMRA, 2001).

Even though an applicant may claim a "narrow host range" for a given mBCA, only directly related species/genera may have been tested to support this claim. Radial taxonomic testing allows for a broader consideration of NTOs (non-target organisms) both closely and more distantly related to the target organism, particularly where there is or may be a shared mode of action (*e.g.* receptor type), a shared behaviour, a similar or likely exposure scenario, and/or environmental/biodiversity value (*e.g.* natives) of a related species.

Radial taxonomic testing is a useful tool for assessors in that it helps to focus the testing of NTOs in those taxonomic groups that are most likely to be affected by the mBCA. These may be groups that fall outside the standard test organisms (*e.g.* rainbow trout, *Daphnia*) recommended under current OECD guidelines for the testing of chemicals. At least, if testing is not conducted, it alerts the assessor to those NTOs most at risk from the mBCA and appropriate responses can be considered (*e.g.* imposing management conditions on the release).

2. Application type and pattern (BOX 2 of decision scheme)

The pattern of use is a very important part of the environmental risk assessment, as it primarily determines the (potential) extent of exposure. It comprises:

- the application rate expressed in CFU/ha (or other relevant units such as granules/ha);
- the frequency;
- the site (crop, bare soil, slope);
- the time (early or late in the crop; early morning or late evening: this informs exposure assessment depending on NTO activity);
- type of application (spray, drip, aircraft, ground equipment).

It should be noted that mBCAs require very specific (micro-) conditions and without these requirements the efficacy is limited. In view of these limitations, biopesticides may be applied more frequently than their chemical counterparts.

In general, only the exposure of NTOs due to outdoor application (*e.g.* spray application, granules, seed treatment) should be considered in the risk assessment. Risk assessments should be based on the specific mBCA considering the intended use (soil or foliar applications), target pest (fungicide, insecticide etc.) and mode of action (pathogen, competition for space and nutrients etc.).

2.1 Indoor

Currently, there is no agreement on the definitions of individual protected/covered crop systems like a specific type of glasshouse. In view of this paucity and uncertainty, the EFSA Panel on Plant Protection

Products and their Residues (PPR) held a workshop in Parma (Italy) on November 17-19, 2009 to discuss the development of a new Guidance Document on emissions of plant protection products from protected crop systems such as glasshouses and crops grown under cover (EFSA, 2010). For microorganisms, exposure routes and amounts may be completely different, if relevant at all. However, for the time being, the outcome of this EFSA Guidance Document may be considered for the risk assessment of mBCAs. Exempting data requirements is not recommended by Canada. Instead, Canada recommends that sound scientific rationales be considered in lieu of data to ensure that no potential risks exist for the mBCA in question.

Types of application and expected exposure of NTOs:

- Indoor use *e.g.* mushrooms, harvested crop:

  no or negligible exposure

- Indoor glasshouses

  Exposure:

  - Emissions due to spray drift from permanent structures via open windows and openings can be considered negligible.
  - Exposure of birds, mammals, aquatic organisms, earthworms, soil microorganisms may not be relevant.
  - Exposure of pollinators such as bumblebees and beneficial arthropods such as predatory mites and parasitic wasps need to be considered as they may be used as part of IPM in combination with mBCAs. Exposure of non-target insects that invade the glasshouse through open windows is considered not to be relevant.
  - Discharge to surface water needs to be taken into consideration.

2.2 Outdoor

At the start of the risk assessment scheme, the NTOs that will likely be exposed in consideration of the application type, need to be determined.

Types of application and expected exposure of NTOs:

- Spray applications to bare soil

  Exposure:

  - Birds and mammals
  - Non-target soil dwelling arthropods
  - Earthworms and microorganisms
  - Emerging plants
  - Aquatic organisms (fish, algae, crustaceae, aquatic invertebrates, aquatic plants)
  - Other NTOs potentially exposed are reptiles and amphibians

- Spray applications to crops (plants and soil)

  Exposure:

  - Birds and mammals
  - Bees; only in flowering crops [or other pollen producing plants (*e.g.* gymnosperms)]
  - Non-target arthropods (soil and plant-dwelling)
  - Earthworms and microorganisms
  - Plants
  - Aquatic organisms (fish, algae, crustaceae, aquatic invertebrates, aquatic plants)
  - Other NTOs potentially exposed are reptiles and amphibians

- Seed treatments (also relevant for bulbs and potatoes)

  If an mBCA is applied to seeds by drench, an exposure of NTOs might occur in the soil. Initial PECsoil should be based on the calculation of the number of CFU of the mBCA per seed multiplied by the number of seeds, bulbs or potatoes per hectare. This will result in local exposure of the rhizosphere of the roots of the plants emerging from the seeds, bulbs or potatoes. The mBCA will multiply and grow along with the newly formed hair roots. In arable fields drilled with treated seeds, a certain percentage of unburied seeds can be expected whereas the availability of unburied seeds depends on the used drilling-technique and other agricultural practices such as seeding depth and soil conditions (de Snoo and Luttik, 2004). Depending on the amount of spillage, risks to seed eating birds and mammals should be taken into account.

Exposure

- Soil arthropods in the rhizosphere
- Earthworms and microorganisms in the rhizosphere
- Granivorous birds and mammals (exposure due to ingestion of treated seeds remaining on the soil surface following drilling or ingestion of spilled seeds).

Situations less likely but to be aware of:

- If the microorganism is able to grow endophytically, an exposure of NTOs might also occur aboveground on plant-dwelling arthropods.

- Point applications: tree injection or as a rub on trunks

As trunk treatment is a very specific local application to a limited area, exposure of the terrestrial compartment could be considered minimal or negligible.

Situations to be aware of:

- Tree injection will not lead to further exposure of the environment, unless the mBCA will grow into the rhizosphere or will sporulate on the leaves. Example: Tree injection of *Verticillium albo-atrum* isolate WCS850 used as vaccine in order to prevent Dutch elm disease.
- There are known cases where fungal preparations applied to the surface of cut wood can sporulate and spread to nearby trees.
- Example: *Chondrostereum purpureum* on black cherry (De Jong et al., 1996).

- Spraying from aircrafts

Exposure:

- In general, all compartments will be exposed (air, surface water and soil) although some compartments will not be exposed under specific conditions [*e.g.* aerial spraying against the desert locust (*Schistocerca gregaria*) in the desert is unlikely to expose water compartments]. Mitigation options are possible (*e.g.* no-spray zones, crop stages, time of day). The only difference in the risk assessment compared to land-based applications is that the relative exposure values for the compartments will be different.
- Other formulation types than spray solutions can be used for aerial spraying (*e.g.* solid pellets), which have other consequences for exposure of environmental compartments, *i.e.* no crop interception, no exposure of air.

3. Fate and behaviour (BOX 3 of decision scheme)

Comparison of data requirements and risk assessment approaches within the OECD

Regulation (EU) No 544/2011 states that experimental data on fate and behaviour are normally required unless it can be justified that an assessment can be performed with the information already available from the open literature for the respective environmental compartment. The respective paragraph 7.1 states as follows: "Where relevant, appropriate information on the persistence and multiplication of the microorganism, in all environmental compartments has to be given, unless it can be justified that exposure of the particular environmental compartment to the microorganism is unlikely to occur." (see also paragraph 1.3.1.1).

The US and Canadian approach, in contrast to that of the EU, does not generally require formal environmental fate data for the reason that the fate and behaviour of a mBCA is difficult to evaluate due to the potential for microbial growth under suitable environmental conditions. Instead of requiring environmental fate data, the US and Canada follow a tiered approach as described in chapters 4.1.2 and 4.1.3.

Accordingly, the need for environmental fate testing depends on the occurrence of detrimental effects in the first tier. Moreover, the Canadian registration guidelines (PMRA, 2001) state that the extent of environmental fate testing is mainly based on the nature of the mBCA, *i.e.*, whether it is indigenous or non-indigenous to the ecozone(s) of intended use.

General options for waiver

In the EU, waivers are accepted if exposure of the NTO can be excluded by the type of application. Waivers are also accepted when significant information about the mBCA, *e.g.* in-depth knowledge of the biology, life-cycle, mode of action, fate and behaviour in the considered environmental compartment is available. The rationale should include a transparently conducted scientific literature search for published pathogenic/toxic effects to NTOs of concern. In this context it should be mentioned that EFSA has recently published a Guidance Document entitled "Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009" (EFSA, 2011). This guidance document shall ensure methodological rigour and transparency, and aims to minimise bias in the identification and selection of scientific information in dossiers. This EFSA guidance is compatible (*e.g.* in terms of format) with existing EU and OECD Guidance documents that are widely used to assist the preparation of dossiers (EC, 2005b; OECD, 2005, 2006).

3.1 Fate and behaviour in the soil compartment

3.1.1 Fate of inoculum; multiplication, accumulation in soil

In Commission Regulation (EU) No 544/2011, annex part B (EU, 2011) the data requirement on fate and behaviour specifically asks for information on 'persistence and multiplication'. Ideally, growth of an "indigenous" mBCA should, after a short growth period, level off, and continue along the line of the background microorganisms. If the application of a mBCA is not expected to increase the natural "background" levels of the species or related species, risks may be considered acceptable or "not deviating" from "normal".

It should be evaluated on a case-by-case approach whether the mBCA, based on its identity and characterization, is likely to survive in the soil. This approach is particularly important for mBCAs that consist of non-indigenous microorganisms, *i.e.* strains and/or species that are not found in the natural environment where the mBCA will be applied. For non-indigenous mBCAs fate/survival tests and NTO testing may be particularly important. However, as stated in the EU Guidance Document SANCO/10754/2005 (EC, 2005a), due to large possible ranges of the environmental factors, data on fate and behaviour of the microorganisms will inevitably show large variability. Therefore, this variability caused by environmental factors could be larger than the possible differences between strains of the same species. On the other hand, the EU Guidance (EC, 2005a) also proposed that data on strains should be treated separately if there is sufficient evidence that strains differ in their environmental fate and behaviour.

For entomopathogenic fungi *B. bassiana, B. brongniartii,* and *M. anisopliae*, data on natural concentrations in the soil and persistence following applications to the soil have been collected in a study performed by Scheepmaker and Butt (Scheepmaker & Butt, 2010). This review is freely available on the OECD site. In this review, a methodology was suggested to determine the natural background level.

Methodology on how to determine the natural background level in three steps

Step 1) Determination upper natural background level

Studies on natural background concentrations were collected from the literature for each of the three species. Those studies were selected that gave at least three data points from one sampling area. The overall geometric mean was calculated for the selected studies as the average of the individual log observations. The overall geometric mean was then the exponent of this value. The derived 95th percentile of the geometric mean was chosen to represents the upper natural background level. By choosing the 95th percentile some very high peaks were excluded. For *M. anisopliae, B. bassiana* and *B. brongniartii*, the upper natural background level was approximately 1000 CFU/g soil. It was clear that natural background concentrations are variable and depend on land use, climate, soil and other possible factors.

Step 2) Collection of fate/survival data of applied inoculum

Studies on survival of applied inoculum were collected from the literature for each of the three species. Despite the variety in between the experiments (length, number of sampling during the course of the experiment, soil, crop, etc.) data from the different sources showed a decline in density for the three fungal species. This decrease was similar for laboratory experiments, small-scale experiments and field experiments.

Step 3) Determination of the time needed for applied inoculum to decrease to upper natural background level

It was graphically estimated that the applied inoculum density decreases to upper natural background levels within 0.5-1.5 year for *B. bassiana*, after about 4 years for *B. brongniartii* and >10 years for *M. anisopliae*.

Conclusions for the risk assessment:

- The review of Scheepmaker and Butt (2010) (see link on OECD site) can be referenced in a waiver/statement to fulfill the persistence in soil data requirement for the three EPF species*, B. bassiana, B. brongniartii, M. anisopliae*. Since other EPF species are subjected to the same processes in the soil, it is assumed that a similar decrease of inoculum will occur in other EPF species. Therefore, the proposed methodology can be used for other mBCAs as well.

- This review showed that applied inoculum of the three EPF species decreases to natural background levels in time and that increases of the inoculum are only temporary and depend on the presence of a population of host insects in the field.

- A wide variety of factors explaining the decline of EPF density was described.

  Some general situations with a negative impact on the survival and fate of the inoculum:

  - the microorganism is subject to competition and parasitism of the autochthon microbial community.

  - the microorganism is subject to predator pressure.

  - the microorganism does not germinate and/or proliferate/or multiply in the soil due to very specific (micro-) conditions.

  - it cannot readily gain energy from hardly degradable substances of limited biodegradability like lignin.

- It is not feasible to collect a set of background studies that are similar regarding soil condition, strain, country, crop, etc., for the simple reason that the data in the literature are not uniform and may be very limited. Moreover, in most cases, studies from the literature are not based on the desired strain for authorization, as these strains often originate from a specific isolate and can therefore only be found in a certain area. For these reasons, it is not feasible to develop standardised methods specifying the minimum number of different conditions, soils, application timings and samplings. This approach is not practicable and too costly.

- Although species potentially differ in toxicity at the strain level, it is recommended to evaluate persistence at the species level as it was shown by Scheepmaker and Butt (2010) since densities of individual strains often follow a very similar decline.

- It should be realized that reproduction of an entomopathogenic fungus may occur in the presence of the host. If occurring, the PEC may increase during a short period of time. After this period, a steady decline of the inoculum is expected to occur.

- In contrast to the criterion of persistence for chemicals, there is no criterion for persistence of mBCAs. From this follows that the length of the period that the applied concentration is higher than the upper background concentration is to be discussed case-by-case. This is clearly the case for *B. brongniartii* and *M. anisopliae.* In general, the persistent mBCA may be present in an inactive state, probably in a patchy distribution confined to small pockets in the soil. The mBCA may be activated under very specific conditions.

3.1.2 Estimation of PEC soil (BOX 4 of decision scheme)

PEC-in crop

For the sake of consistency the widely used term "PEC" (predicted environmental concentration) is considered in the present document to express (quantify) exposure level, although the term "PED" (predicted environmental density) may be regarded as more appropriate in ecological terms.

In general, it should be taken into consideration that for many biological products based on microorganisms the active ingredient is very susceptible to UV light, dry conditions etc. For this very reason many products need to be applied at a regular interval.

It was agreed in the EU Pesticide Risk Assessment Peer Review (PRAPeR) of plant protection products (PPP) containing microorganisms to estimate the PECsoil by assuming a density of the soil of 1500 kg/m^3 and a distribution in the soil in the top 5 cm. This approach is in line with PECsoil calculation of chemical substances. This estimate is conservative as the actual concentration in soil after application is always lower than predicted due to loss in viability of the mBCA (with the exception of a possible short period of increases (*e.g.* reproduction of entomopathogenic fungi in hosts)). It is more difficult to determine the concentration (population density) of a living organism in soil compared to a chemical substance. An appropriate measure of mBCAs in soil would be to estimate the predicted initial concentration in soil (PIEC) using the summation of the nominal concentrations used in the repeated applications. These PIECsoil values should be compared to background concentrations if available. With molecular techniques it is possible to determine the exact concentrations in the soil. Other approaches are available: Environment Canada uses a model into which degradation of inoculum is integrated. This model assumes distribution in the upper 15 cm of the soil.

Crop interception values should be included in the estimation of the PEC values. Care has to be taken when using the interception values of chemicals as these are not validated for microorganisms. Nevertheless, the interception of a crop can be included in the calculation when the application technique (spray equipment) and formulation (additives, spreader etc.) are similar or identical to chemical products. If interception would not be considered for the calculation of the PEC values then risk evaluation would be done on full accumulated PEC values on crop and in soil which would not be equivalent to the risk assessment of chemicals.

PEC-off crop

Canadian drift model(s) employed to determine off-crop exposures needs to calculate deposition from different spray methods and equipment (*e.g.* ground boom, airblast and aerial). Calculating a PEC off-crop value would only be necessary if exposure of NTOs requires refinement due to adverse effects noted at the Maximum Hazard Concentration (MHC) and Maximum Hazard Dose (MHD), respectively.

Moreover, the expected concentrations are below the levels tested in the worst case scenario in Tier I studies. Therefore, side effects are not to be expected. mBCAs are sensitive to UV-light, desiccation and other abiotic factors, therefore any CFU (microbe) deposited by spray drift has hardly a chance to survive.

3.2 Fate and behaviour in the aquatic compartment

3.2.1 Estimation of PECsw, MoS and MHC (BOX 4 of decision scheme)

- ***PECsw***

In the EU PRAPeR Expert Meeting M2 held on 16-18 February 2009, it was proposed that, due to lack of appropriate methods, initial exposure in surface water can be calculated using the Ganzelmeier drift tables leading to PEC values (Ganzelmeier *et al.*, 1995, updated by Rautmann *et al.*, 2001), whereas entry paths such as runoff, drainage and aerial deposition require different approaches. It was concluded that exposure estimation has to be solved on a case-by-case basis. The above mentioned EU PRAPeR proposal using initial PEC values can be regarded as a conservative approach since the actual concentration following application is likely to be lower than the predicted concentration as many environmental parameters cause loss of viability of the mBCA within a relatively short time frame. Also, the mBCA may (in some cases) quickly precipitate to the sediment in calm water, leading to lower concentrations distributed throughout the water. In the latter case, effects on sediment organisms may be required provided that the mBCA has the potential to adversely affect invertebrates. Therefore, for risk assessment purposes, particular attention should be paid to environmental conditions affecting viability of mBCAs. Information should be available on the fate of the mBCA in surface waters with various oxygen conditions, sensitivity to solar radiation and its influence on growth and germination capability. Based on the outcome of the 4th stage EU review programme containing microorganisms, there is an increasing body of evidence suggesting that most mBCAs are not viable in non-sterile water due to competition with other microorganisms or due to unfavourable environmental conditions. Therefore, long-term exposure in surface water is expected to be unlikely while acute and short-term exposure cannot be excluded.

- ***MoS***

Margins of safety (MoS) between the units of microorganisms per ha on the one hand and toxicity values on the other hand can be derived. As a general conclusion, a rough estimation of the initial concentrations seems appropriate in the first Tier level.

- ***MHC***

Canada does not routinely apply spray drift models to assess drift and off-target exposure potential of mBCAs. The Ganzelmeier drift tables have been considered in Canadian spray drift models for ground application of conventional chemical pesticides (http://www.hc-sc.gc.ca/cps-spc/pest/agri-commerce/drift-derive/index-eng.php, last accessed, April 27, 2011). For mBCAs, Canada, however, recommends that each mBCA be considered separately based on its own biological properties as well as its proposed use pattern. The MHC approach (Tier I), in Canadian opinion, is the simplest approach since it eliminates the requirement for fate testing and focuses on potential hazards, *i.e.*, assumes that NTOs may be exposed.

According to the US EPA and Canadian approaches a MHC for surface water of 10^6 CFU/mL or 1000 times the expected microbial concentration in water bodies is defined. In order to determine the MHC or simply to judge whether the MHC is high enough (margin of safety) the setting of the MHC needs to be verified prior testing.

4. Environmental toxicity (BOX 5 of decision scheme)

The established European environmental risk assessment for chemical pesticides is primarily based on the calculation of the quotient between ecotoxicological endpoints (ER_{50}, LD_{50} or NOEC) and predicted environmental concentrations being the TER-value. This approach has also been used by some European member states compiling draft assessment reports (DARs) for List III and IV mBCAs. These calculations served mainly as an approximate assumption of the relation between endpoints gained from submitted toxicity/pathogenicity studies and estimated environmental concentration (or environmental density). The estimated environmental exposure were mostly calculated by using methods being developed for chemical pesticides, as no specific exposure models for mBCAs are available so far. These calculations were accompanied by further qualitative statements leading altogether to a semi-quantitative risk assessment.

In fact, exposure scenarios for chemicals are not fully applicable to mBCAs. The maximum hazard concentration (MHC) or maximum hazard dose (MHD) [note: the synonym "Maximum Challenge Concentration (MCC)" is being used in the Canadian Guidelines] circumvents this problem by using the maximum amount of active ingredient (mBCA or its toxin) in the toxicity/pathogenicity studies. A definition of the MHC/MHD to be used can be found in each guideline. As the MHC/MHD approach shall cover anticipated exposure levels including a safety factor, no further risk calculation is necessary in most cases. It goes without saying that the MHC/MHD needs to be carefully established. For example for EPF or viruses, the Canadian guidelines (Environment Canada, 2004) propose additional safety factors due to their potential multiplication: "For mBCAs that are expected to increase significantly in the environment following an application, *e.g.* viruses in insects, the oral dose administered should be no less than the highest concentration possible in field, *e.g.* equivalent to the numbers in maximally infected insects".

The amount of studies required for the risk assessment may depend on various factors and properties of the mBCA. For instance, production of bioactives (toxins, metabolites) might affect data requirements and should be considered in the following way: If metabolites/toxins are known to be responsible for the mode of action, and if there is relevant exposure of NTOs then, in that case, toxicity data should be available and a risk assessment for the metabolites/toxins of ecotoxicological concern should be performed. Currently, Butt and Scheepmaker are working on a project that categorises metabolites/toxins and their effects on NTOs using information that is available in scientific literature. Risk strategies will be proposed. It is anticipated that this work will be transformed into OECD guidelines for assessing the risks of fungal metabolites.

4.1 Test Guidelines

4.1.1 OECD Guidelines

Within the EU, standard OECD tests are available to determine the potential toxic effects of (chemical) pesticides. The advantage of studies according to the OECD guidelines is the determination of LD_{50}, ER_{50} values due to the usage of numerous test concentrations. These OECD guidelines are considered to be less appropriate for assessing possible effects of mBCAs to NTOs since the recommended duration of these tests might not be sufficiently long to allow infection. Furthermore, some expected routes of exposure are not considered in all cases.

4.1.2 Data requirements and risk assessment according to OCSPP Guidelines of US EPA

US EPA does not require dose-response testing in the first Tier level. OCSPP guidelines give the option of testing a single group of test species at the MHD (maximum hazard dose), thus giving a No Observable Adverse Effects Limit. Test Guidelines are available on the website: http://www.epa.gov/ocspp/pubs/frs/publications/Test_Guidelines/series885.htm (last accessed, April 27, 2011). The MHD for Tier I testing will be based on a safety factor times the maximum amount of active ingredient (mBCA or its toxin) expected to be available to terrestrial and aquatic plants and animals in the environment. These Tier I studies are of sufficient duration (*i.e*., typically 21 to 30 days) to increase the likelihood of detecting any adverse effects due either to toxicity or infectivity/pathogenicity and allow for specific routes of exposure. A short summary of the tiered approach is given in Table 1 below.

Table 1: Tiered testing approach by the US EPA

Tier level	Explanation
I	Tier I consists of maximum dose single species hazard testing on NTOs
II	If adverse effects are observed in Tier I, the potential exposure to the MPCA is estimated by means of Tier II testing for population dynamics, fate and expression in the environment
III	If Tier II tests show that there may be significant exposure to the MPCA, Tier III studies to determine a dose response effect or to examine certain chronic effects will be performed to determine if the minimum infective dose is less than the exposure or if there are other considerations that would decrease the observed effects in the environment.
IV	Tier IV tests, under simulated or actual environmental conditions, are to be designed on a case-by-case basis to evaluate any specific problem that cannot be resolved by lower tier testing.

According to the OCSPP approach, dose-response tests are only needed if any adverse effects are observed in Tier I MHD studies. In practice, US EPA never needed microbial pesticide NTO studies rendering an LD_{50}. If unacceptable adverse effects are identified in Tier I tests, Tier II tests are performed attempting to quantify levels of the mBCA to which the susceptible non-target species may be exposed. Tier II Environmental expression testing consists of simulated terrestrial and aquatic applications of the mBCA. Terrestrial and aquatic applications are conducted in a contained environment (greenhouse, aquaria) to assess survival and growth in soil, vegetation, water and sediment. The contained environment in the environmental expression tests are generally based on natural materials from the proposed use site (sediment, soil, plants, and marine/estuarine liquids) which are arranged as naturally as possible, and held within a plastic, glass or other container to prevent escape of the microbial agent.

As a result, Tier II information indicating that the mBCA will not survive or persist in the environment to which it is applied, can be submitted as support for a request for waiver of some or all of Tier I testing requirements. In case of lasting concerns as an outcome of Tier I and Tier II studies, further Tier III (prolonged Tests) or Tier IV (field testing) studies may be required.

4.1.3 Data requirements and risk assessment according to Canadian Test Guidelines

Environment Canada has developed a Guidance Document for testing the pathogenicity and toxicity of new microbial substances to aquatic and terrestrial organisms (Environment Canada, 2004). This guidance document is available on:

http://www.ec.gc.ca/Publications/default.asp?lang=En&xml=F9BF9993-4BAC-4215-BD3E-9B0962980915 (last accessed April 27, 2011).

The Canadian Guidance Document takes into account various sources of information, including guidance in PMRA[2]s microbial registration guidelines (DIR2001-02), US EPA test guidelines and OECD test guidelines. PMRA registration guidelines (Regulatory Directive DIR2001-02, *Guidelines for the Registration of Microbial Pest Control Agents and Products*) provide general guidance on study design and reporting and are available on PMRA's website (http://www.hc-sc.gc.ca/cps-spc/alt_formats/pacrb-dgapcr/pdf/pubs/pest/pol-guide/dir/dir2001-02-eng.pdf, last accessed, April 27, 2011).

According to Canadian registration guidelines (PMRA, 2001) a four-tiered testing approach is followed similar to the approach by the US EPA. However, minor deviations are noted regarding the testing criteria of the NTOs and fate testing procedures (see Table 2 below).

Table 2: Tiered testing approach according to Pest Management Regulatory Agency (2001)

Tier level	Explanation
I	Test organisms in Tier I are exposed to maximum hazard or Maximum Challenge Concentration (MCC) of the mBCA. Criteria for non-target to be tested are as follows: taxonomically related, infected by mBCA, high exposure potential, similar physiology, susceptible to related pathogens, representative species from seven broad taxonomic groups
II	Adversely affected species from Tier I toxicology tests are exposed to Lower Challenge Concentrations (LCC). Conditionally required fate studies are as follows: pure culture testing, microcosm testing, small- or large-scale field studies
III*	Adversely affected species from Tier II toxicology tests are exposed to multiple concentrations (determination of LC50, LD50, EC50 values) Conditionally required fate studies are as follows: small- or large-scale field studies
IV	Adversely affected species from Tier II are investigated in small-scale field studies in which the end-use product should be used.

*Tier III testing is not required for indigenous mBCAs

Overall, data requirements for mBCAs are similar between regulations in Canada and the US with regard to effect analysis, whereas few test systems recommended in the Guidance Document (Environment Canada, 2004) are not usually required in the US EPA Microbial Pesticide Branch such as the 56-day

[2] Note that Environment Canada and PMRA are two different governmental departments in Canada

earthworm reproduction study or the 28-day collembolan reproduction study, respectively. In addition, Canadian guidelines were developed for both single concentration and multi-concentration-tests taking into account maximum hazard testing approach. The Canadian guidelines are more advantageous because they contain more specific statements regarding certain test conditions and test criteria.

4.1.4 Opinion of the BPSG

The BPSG had agreed to use the US Microbial Pesticide Testing Guidelines although Canadian test guidelines may also be used.

4.2 Waiver options

Waivers can be granted in two situations:

1. If exposure of the NTO can be excluded by the way of application.

2. If significant information is available for the mBCA, *e.g.*, in-depth knowledge of the biology, life-cycle, mode of action, fate and behaviour in the considered environmental compartment.

The rationale should include a transparently conducted literature search on the pathogenic/toxic effects to NTOs of concern according to EFSA's Guidance Document on Submission of scientific peer-reviewed open literature (EFSA, 2011).

4.3 Terrestrial NTOs

4.3.1 Birds and mammals

a) Birds

Available test guidelines

1) US EPA test guidelines OCSPP 885.4050 Avian Oral Tier I.

In this study, a MDD (maximum daily dose) is administered to young bobwhite quail or mallard ducks for five days with a following observation period of at least 25 days. If any signs of pathogenicity and toxicity are manifested on the 30^{th} day, observation should continue until recovery, mortality or unequivocal moribundity is established.

The highest oral dosage level tested is defined by the following formula:

$$\text{MDD (units)} = [\text{mBCA}] \text{ in TGAI} \times 5 \text{ mL/kg BW} \times \text{ weight of test bird (kg)}$$

where
[mBCA] = concentration of mBCA
TGAI = technical grade of active ingredient
BW = body weight

The treatment group is accompanied by three different control groups:

- A negative, non-dosed control group.
- An infectivity control group treated with the mBCA inactivated in such a way as to retain the structural integrity of the cell.
- A control group in which the birds are dosed with sterile filtrate from production cultures.

2) The test guidelines recommended in the Canadian guidance document are consistent with the US EPA test guidelines. The Canadian proposal for test method provides additional criteria of validity (invalid if < 90 % survival in negative control at test end), a more detailed description of conducting a multi-concentration test and the requirement for assessing infectivity at the end of the test (as a minimum).

b) Mammals

Available test guidelines

1) US EPA provides specific test guidelines for detecting effects in wild mammals (885.4150). Overall, results of the toxicology studies might be sufficient to address possible adverse effects to mammals.

Mammals feeding on insects infested by entomopathogenic mBCAs

Animals feeding on insects can be expected to ingest large quantities of actively growing microorganisms when they feed on diseased insects. Moreover, there is a possibility for exposure to potential toxic secondary metabolites synthesized during vegetative growth. This issue is addressed in Tier I freshwater fish testing (OCSPP 885.4200) since there is an option of exposing fish with infected insects. However, no test guidelines are available to address this type of exposure in insectivorous birds and mammals.

Risk assessment

If no negative impact to birds and mammals are observed in the study, the risk can be considered to be acceptable.

If the MHD study shows negative effects, an attempt to classify the type of effect(s) observed in the study should be made by using observations of pathogenic symptomatology or pathological changes, gross necropsies and histopathological findings, and by comparing these observations to those made for the various control group(s) (*e.g.* non-infectious control and sterile filtrate control).

Toxic effects:

If the observed effects are mainly attributed to compounds with a toxic mode of action, *i.e.* caused by toxic co-formulants or other components of the technical material (probably secondary metabolites resulting from the microbial growth in the batch of production), a dose-response study should be conducted to obtain reliable ecotoxicological endpoints such as $LD_{50}/LC_{50}/EC_{50}/ER_{50,}$ NOEC or NOEL values. Consequently, a standard risk assessment comparable to chemical pesticides including standard safety factors might be feasible. In accordance with the Regulation (EC) No 1107/2009 of the European Parliament and of the Council and the Guidance Document on Risk Assessment for Birds & Mammals (EFSA , 2009), a TER calculation can be conducted taking into account Annex VI TER trigger values of 10 and 5 for acute and chronic exposure, respectively.

As the toxicity causing metabolites are probably unknown, derived ecotoxicological endpoints must refer to the amount of technical material or to the units of the mBCAs itself.

Pathogenic effects:

If the adverse effects are caused by pathogenicity, a follow-up with dose-response testing might not always be appropriate since clear dose-response relationships may not necessarily be observed.

Information on growth-temperature-relationship of the mBCA

The ability of an mBCA to grow at body temperatures can be regarded as a crucial factor in the evaluation of pathogenic effects.

As stated in the Commission Regulation (EU) 544/2011 (EU, 2011) in annex part B point 2.5 (Infectiveness, dispersal and colonisation ability), the temperature range at which the mBCA grows must be determined, including minimum, maximum and optimum temperatures. This information is of particular value as a trigger for effect studies on human health. It is also, to some extent, of importance for evaluating effects on birds and wild mammals. However, it should be emphasised that a single statement "inability of growth at temperatures of >36°C" should not be accepted as a waiver. Nevertheless, adequate information on growth-temperature characteristics together with additional arguments might be acceptable. Further, unnecessary testing of vertebrates should generally be avoided in view of animal welfare.

Incidental remarks on vertebrates other than birds and mammals:

It is obvious that information on the growth-temperature characteristics of mBCAs do not provide a strong waiver argument for cold-blooded animals such as amphibians and reptiles. Given the lack of microbial-specific test methods for amphibians/reptiles and because they are not formally required for conventional pesticides (except the Metamorphosis Assay with the African clawed frog *Xenopus laevis* in case of thyroid active substances), effect studies with (amphibians/reptiles) should only be provided on a case-by-case basis, *e.g.,* if susceptibility is reported in the open literature, or there is strong evidence for adverse effects based on the biological properties of the mBCA.

Waiver options

A waiver can be submitted:

- If exposure of birds and mammals is expected to be minimal or negligible.
- If significant information is available for the mBCA, *e.g.*, in-depth knowledge of the biology, information on growth-temperature characteristics, life-cycle, mode of action, fate and behaviour

in the considered environmental compartment must be submitted based on a transparently conducted literature research for published pathogenic/toxic effects to birds/mammals.

A waiver can be granted if sufficient information is available to conduct a qualitative risk assessment and thus in logical line of argument a potential risk of the mBCA to birds/mammals can be excluded.

4.3.2 Bees

General aspects

The European Regulation (EC) No 1107/2009 requires that the risk to bees be evaluated. The US EPA as well as Canada only provide guidelines for testing honey bees.

Available test guidelines

1) OCSPP guidelines 885.4380 Honey Bee Testing Tier I

- No MHD is defined.
- On the basis of 885.4340 (Nontarget insect testing) dosage shall be in suitable increments of up to 100 times the LD_{50} or LC_{50} of the pathogen in its natural host, or 10–100 times the recommended field dosage.
- The method of application depends on the expected route of exposure, either oral or contact or even whole-hive.
- The recommended test duration is ≥ 30 days. When the mBCA may be expected to affect larvae, honey bee larvae should be included as test organisms.

2) Honeybees Acute Oral/Contact Toxicity Tests (OECD 213/214) (Tier I)

- Test duration is 2 days which is too short to measure pathogenic as well as toxic effects. Therefore they do not provide an acceptable alternative to OCSPP guidelines 885.4380.

3) A standardised method by Aupinel *et al.* (2005)

- This method can be recommended for assessing the risks to bee brood.

4) EPPO 170 guidelines (EPPO, 2010)

- Additional long-term (semi)-field testing may also be required if effects are observed in the first tier level.

Additional information on laboratory test methods is given below:

According to the Canadian guidance document (Environment Canada, 2004), US EPA is undertaking research studies with the intent of developing a standardised laboratory test method for measuring the ecological effects of microbial substances on honey bees. The publication of Hanley *et al.* 2003 (cited in Environment Canada, 2004) describes a laboratory test used to demonstrate the potential adverse effects of dietary pollen contaminated with microbial or chemical pesticides on larval or pupal life stages of honey bees. Certain aspects of the test design including larval and pupal mortality rates as well as reduced pupal weights as biological endpoints are being considered by the US EPA for possible use when developing a standardized protocol.

The Canadian guidance document mentions two other published reports on laboratory tests performed with groups of adult honey bees. Ball *et al.* 1994 (cited in Environment Canada, 2004) acclimated groups of young adult honey bees (25/cage) to laboratory conditions in cages for 1 week, followed by their exposure to a mycopesticide administered by spray application. The negative control group showed a mortality rate of only 7 % during a subsequent 12-day period of observation. Butt and Goettel (2000, cited in Environment Canada, 2004) using a similar experimental design did not report the mortality rate of control groups, although a 14-day mortality of only 11 % was found for groups of adult bees subjected to the lowest microbial test concentration tested, with higher mortality rates (up to 87 %) for higher concentrations. Both research studies indicate that acceptably low (*e.g.* ≤ 10 %) mortality rates can be achieved for negative control groups of adult honey bees, in 12-14-day laboratory test using this experimental design. The Canadian guidance document considers both approaches as promising, but recommends for applying this test design to conduct preliminary tests of 14 day duration to ensure that an acceptable control mortality rate of ≤ 10 % can be achieved. Additionally alternatives for dosing the test groups by feeding them a diet containing the microbial substance or by spray application should be considered and experimented with in preliminary trials.

Risk assessment

The calculation of HQ values as used for chemicals (application rate/LD_{50}) is generally regarded as less feasible for risk assessments with mBCAs because dose-response relationships are rarely observed in cases of pathogenic effects.

If observed effects are caused by toxicity, dose-response testing should be conducted providing reliable ecotoxicological endpoints ($LC_{50,}$ LD_{50}, NOEC). These endpoints can be integrated in standard risk assessments similar to chemical pesticides including the use of established safety factors. Exploring the origin of effects observed in control groups receiving an attenuated treatment (microbe-free or non-viable microbe comprising material from the culture system used for propagation) might be helpful, as adverse effects caused by this treatment are not due to pathogenicity.

According to the US EPA guideline OCSPP 885.4340, the MHD is up to 10–100 times the recommended field dosage, thus comprising a safety factor of 10–100. According to Environment Canada (Environment Canada, 2004), "the maximum hazard concentration is to be equivalent to 100 times the maximum concentration of microorganisms specified by the notifier for the final tank mix of a microbial

product, when it is applied at the maximum label rate". A subsequent risk calculation requiring an adequate exposure assessment is not necessary provided that no adverse effects are observed.

In instances of pathogenic effects, these observations have to be considered and classified in context of the overall-knowledge about the mBCA (physiological and ecological host range, mode of action, life-cycle and biology of the mBCA, environmental conditions for survival, germination and infection).

Since bumble bees have a far lower hive temperature compared to honey bees, they might be more susceptible to mBCAs, particularly to EPFs having mostly lower optimal growth temperatures (Hokkanen *et al.*, 2003). This should be taken into consideration. Additional study guidelines adapted to bumble bees could be helpful.

Waiver options

A waiver can be submitted:

- If exposure of bees is negligible or minimal.
- In case of non-entomopathogenic mBCA, if database searches find no reports of detrimental impacts of the considered microorganisms on bees and other closely related species of the mBCA that share the same ecological habitat.

4.3.3 Non-target arthropods other than bees

a) Leaf-dwelling arthropods

General aspects

mBCAs can be applied to control fungal or bacterial plant diseases, weeds or pest insects. In the control of insect pests, non-target arthropods (NTA) are the organisms that are most at risk, being relatively closely related to the target organism. Many microorganisms exert their effect(s) through pathogenicity as well as toxicity.

The European regulation does not distinguish between soil- or leaf dwelling arthropods.

Nevertheless, a differentiation between soil and leaf dwelling arthropods is useful as tests with leaf-dwelling arthropods can be waived if exposure can be excluded due to application techniques (*e.g.* soil drench application) and due to formulation types such as granules, seed treatments and pellets (please note: as pointed out in chapter 1.2.2, exposure of plant-dwelling arthropods, in theory, might also occur following seed treatment uses if the microorganism is able to grow endophytically.)

In the US, arthropod tests are only required if the mBCA is intended to control target insect pests by a mechanism of infectivity (*e.g.*, may create an epizootic condition in non-target insects).

Available test guidelines

To date there are no internationally recognized standard test methods for testing the effects of microbials on non-target arthropods comparable to existing OECD Test Guidelines.

Current test guidelines used for evaluating chemical pesticides are only suitable to a limited extent.

1) The IOBC Test Guidelines by Candolfi *et al.* (2000) are generally used to determine side-effects of chemical pesticides on a large range of beneficial arthropods (natural enemies) including both plant-dwelling (*e.g.* the parasitic Hymenoptera *Aphidius rhopalosiphi*, the predatory mite *Typhlodromus pyri*) and ground/soil-dwelling arthropods (*e.g.* the carabid beetle *Poecilus cupreus*, the rove beetle *Aleochara bilineata* and the wolf spider *Pardosa* spp.). However, these guidelines do not consider relevant routes of exposure for viruses, fungi and bacteria, nor do they allow for prolonged exposure and observation periods.

2) The OCSPP guidelines 885.4340 (Non-target Insect Testing Tier I) provides guidance on developing suitable test designs.

   - *Test species*: Three species of arthropods have to be chosen from at least two identified groups (i.e. parasitic Diptera, predaceous Hemiptera, predaceous Coleoptera, predaceous mites, predaceous Neuroptera and parasitic Hymenoptera).

   - *Route of exposure:* Route of exposure should be consistent with the most likely route of exposure under natural environmental conditions. Exposure in the diet is mostly preferred. Considering viral and bacterial mBCAs, these guidelines recommend that internal parasites be tested with virus/bacteria-infected hosts or if they can be cultured *in vitro*, the virus/bacteria can be added to the diet. External stages of parasites and predators (if they are obligatory) may be fed virus/bacteria-infested hosts, virus/bacteria-contaminated media, or virus/bacteria suspended in sugar or honey solutions. The exposure of fungal mBCAs should simulate field conditions as much as possible. Humidity might be critical during exposure.

   - *Test concentration*: The test dosage shall be in suitable increments up to 100 times the LD_{50} or LC_{50} of the pathogen in its natural host, or 10–100 times the recommended field dosage.

   - *Control*: A concurrent control group treated with microbe-free (or non-viable microbe) material from the culture system used for propagation is recommended.

   - *Observations and biological endpoints:* Mortality and symptoms of pathogenicity are to be determined

   - *Test duration:* Test duration depends on the type of microorganism under investigation as well as on the host species and life stage, 8–10 days for fungi, 21–30 days for bacteria and up to 30 days for viruses or until control mortality rises up to 20%.

3) The Canadian guidance document (Environment Canada, 2004) also gives useful advice on designing tests. In chapter 13.3.1, several protocols for testing the effects of microbial pathogens on non-target beneficial insects and mites are mentioned.

   1. Methods for testing the pathogenicity and virulence of fungi on:

   - the predatory mite *Metaseiulus occidentalis* (Sewall and Lighthart, 1989, cited in Environment Canada, 2004)

   - the parasitic wasp *Trichogramma pretiosum* (Sewall and Lighthart, 1990, cited in Environment Canada, 2004)

- the green lacewing *Chrysoperla carnea* (Donegan and Lighthart, 1990, cited in Environment Canada, 2004)

- the lady beetle *Hippodamia convergens* (James and Lighthart, 1992, cited in Environment Canada, 2004)

2. Test for pathogenicity and virulence of bacteria on:

- the lady beetle *Hippodamia convergens* (James and Lighthart, 1990, cited in Environment Canada, 2004)

  Exposure is generally achieved by dipping the test insects in different concentrations of test material followed by observation periods of 6 to 10 days and, if possible, the determination of LD_{50} values.

  The Canadian guidance document (Environment Canada, 2004), as opposed to US EPA guideline 885.4340, recommends that the biology of the microorganism (*e.g.* known pathogens in the same family or genus) be considered during the selection of a suitable test organism and the associated biological test method. If closely related microorganisms are pathogenic to any terrestrial invertebrate, this species of invertebrate should be selected as a test organism, provided that a suitable biological test method is available. The use of computerized databases with a focus on environmental safety issues of microbial pathogens is recommended to identify arthropods species that may be susceptible to a given mBCA. Database results within the genus of the microorganism should be identified and reviewed.

4) The review by Fisher and Briggs (1992, cited in Environment Canada, 2004) considers a variety of important parameters when testing the effects of mBCAs on non-target insects in the laboratory, including choices of test (host) organisms, various routes of exposure, quantifying the test concentration, test duration and endpoints. Moreover, a brief description of research approaches and (non-standard) test methods for measuring effects of microorganisms on honey bees and other non-target insects is included. The Canadian guidance document regards this publication as helpful when choosing the test method(s) for terrestrial invertebrates to be applied to mBCA.

Considerations to entomopathogenic mBCAs

The Canadian proposal for selecting a suitable test organism might be adapted for entomopathogenic microorganisms, because they are intended to control target arthropods. EPF may have broad host ranges, including non-target arthropods. Thus, a species found to be susceptible in the database might belong to the known host range. In instances where an entomopathogen has a broad host range, some effects on non-target arthropods might occur. In such cases, regulators may have to accept some level of pathogenic effects to non-target arthropods.

Risk assessment

The calculation of HQ values as used for chemicals (application rate/LR_{50}) is generally regarded as less feasible for risk assessments with mBCAs because dose-response relationships are rarely observed in cases of pathogenic effects.

If observed effects are caused by toxicity, dose-response testing should be conducted providing reliable ecotoxicological endpoints (LR_{50}, LC_{50}, LD_{50}, NOEC). These endpoints can be integrated in standard risk assessments similar to chemical pesticides including the use of established safety factors. According to the US EPA guideline OCSPP 885.4340, the MHD is up to 10–100 times the recommended field dosage therefore comprising a safety factor of 10–100. According to Environment Canada (Environment Canada, 2004) "the maximum hazard concentration is to be equivalent to 100 times the maximum concentration of microorganisms specified by the notifier for the final tank mix of a microbial product, when it is applied at the maximum label rate". A subsequent risk calculation requiring an adequate exposure assessment is dispensable provided that no adverse effects are observed.

In instances of pathogenic effects, these observations have to be considered and classified in context of the overall-knowledge about the mBCA (physiological and ecological host range, mode of action, life-cycle and biology of the mBCA, environmental conditions for survival, germination and infection).

It is concluded that detrimental effects to non-target arthropods within the host range (ecological host range) have to be accepted to some extent.

Waiver options

A waiver can be submitted:

- If exposure of leaf-dwelling arthropods is considered to be minimal in view of certain application techniques (*e.g.* soil drench application) and formulation types (*e.g.* granules, seed treatment, pellets).

- In case of non-entomopathogenic mBCA, if database searches find no reports of detrimental impacts of the considered microorganisms together with sufficient information about mode of action, biology, life cycle and environmental conditions for survival and reproduction.

b) Soil-dwelling arthropods

Available test guidelines

1) The US EPA provides no test guidelines concerning soil-dwelling arthropods.

2) Canadian guidelines are available for a 28-day reproduction test using the springtail *Folsomia candida* (Collembola: Isotomidae).

Database searches may lead to other susceptible soil-dwelling arthropods. These should be considered as test organisms provided that any testing method is available or might be adapted (*e.g.* IOBC guidelines) to microbial test substances.

Risk assessment

A risk calculation analogous to chemicals is generally regarded as less feasible for the risk assessments of mBCAs because dose-response relationships are rarely observed in cases of pathogenic effects.

If observed effects are caused by toxicity, dose-response testing should be conducted providing reliable ecotoxicological endpoints (LR_{50}, NOEC). The Canadian Guidelines for *F. candida* provides the option of multi-concentration tests. These endpoints can be integrated in standard risk assessments similar to chemical pesticides including the use of established safety factors. Exploring the origin of effects observed in control groups receiving an attenuated treatment (microbe-free or non-viable microbe comprising material from the culture system used for propagation) might be helpful, as adverse effects caused by this treatment are not due to pathogenicity.

According to Canadian guidelines for testing the springtail *F. candida*, a MHC for soil of 10^6 microbial units/g soil (dry wt) or 1000 times the expected microbial concentration in soil within the terrestrial environment is defined. A subsequent risk calculation requiring an adequate exposure assessment is dispensable provided that no adverse effects are observed. When using other guidelines adapted for microbial test substances, a similar test exposure should be adopted.

In case of pathogenic effects, these observations have to be considered and classified in context of the overall-knowledge about the mBCA (physiological and ecological host range, mode of action, life-cycle and biology of the mBCA, environmental conditions for survival, germination and infection).

Waiver options

A waiver can be submitted:

- If exposure of soil-dwelling arthropods is negligible or minimal

- In case of non-entomopathogenic mBCA, if database searches find no reports of detrimental impacts to soil-dwelling arthropods caused by the considered microorganisms in connection with sufficient information about mode of action, biology, life cycle and environmental conditions for survival and reproduction.

4.3.4 Terrestrial plants

General aspects

Information and/or testing for plant toxicity/pathogenicity is not required according to Regulation (EU) No 544/2011 annex part B.

In contrast to the EU, effects on terrestrial plants have to be assessed in the US and in Canada if the mBCA is closely related to a known plant pathogen.

Diseases of commercially important plants have been intensively studied for decades and many plant pathogens have been identified and subsequently well characterized. Some plant pathogens have a very narrow host range and may attack only one species of plant, other plant pathogens may attack a wide range of plant species, and still other microorganisms have never been identified in association with disease in plants.

Available test guidelines

1) The US EPA guideline OCSPP 850.4300

These guidelines refer not only to terrestrial but also to aquatic plants. As terrestrial plants are discussed in this chapter, only this part of these guidelines is considered here.

- *Test species*: Commercial agricultural crops should be considered as test species since these plants are more susceptible to plant diseases compared to wild plants being genetically diverse groups. Besides US EPA emphasizes their commercial importance. The number of species tested depends on the similarity of the mBCA to known plant pathogens. A rationale for the selection of the species to be tested must be provided. Commercial agricultural crops are recommended as test species are listed in the OCSPP guidelines 885.4300. However it is suggested that depending upon the predicted use pattern, certain forest tree species, ornamental trees and shrubs, and weed species may need testing.

- *Test concentration:* One single concentration level at the "maximum label rate" is to be tested, that means the amount of active ingredient in the recommended quantity of carrier.

- *Control:* Negative (untreated) as well as positive controls have to be included. Positive controls are to ascertain that environmental conditions are such that penetration, infection, and disease development are likely to occur in a susceptible host. Therefore the positive control should be selected to closely resemble the subject mBCA in terms of taxonomy and optimal conditions for infection and disease development, if known. In the case of a mBCA not intended for herbicidal use, the positive control may consist of a known plant pathogen, with taxonomic characteristics similar to the mBCA and its susceptible host. In the case of a microbial herbicide, however, the positive control should consist of the target pest weed and the microbial herbicide.

- *Test duration*: Plants should be observed weekly or more frequently until normal harvest or death, or, in the case of perennials, at regular intervals for at least 2 years.

2) The Canadian guidelines

- *Test species and test method*: various monocotyledons and dicotyledons depending on the intended way of application and the likelihood of exposure (see section 12 of the Canadian guidance document). In order to find appropriate test organisms, all results of previous laboratory tests involving terrestrial plants exposed to the mBCA or ones having similar characteristics should be taken into consideration. All available research findings from relevant experimental field studies should be reviewed and considered. Computerized databases should be consulted as a first step in choosing the appropriate test method and test host species. Besides the mBCA under investigation, all microorganisms within the same genus should be identified and reviewed in the same way. Plants found to be adversely affected by the mBCA or by related microorganisms within its genus should be considered as test species provided that suitable test methods are available.

- *Route of Exposure:* The test plants should be exposed to the mBCA by whatever route of exposure that is expected by the proposed use pattern, *e.g.*, in test water, in test soil, by wounding and spraying. Specific susceptibility of a given life stage, possible ways of entry of the potential pathogen (seed, root, leaf) and controlled climatic conditions are to be taken into account

- *Test concentration:* The MHC is defined for soil as 10^6 microbial units/g soil (dry wt) or 1000 times the expected microbial concentration in soil within the terrestrial environment provided this is readily attainable under laboratory conditions.

- *Control:* An additional control group using the sterile filtrate is recommended.

- *Test duration:* Test duration of only 14 and 21 days, respectively depending on the test plant.

Risk assessment

A risk calculation analogous to chemicals is generally regarded as less feasible for the risk assessments of mBCAs because dose-response relationships are rarely observed in cases of pathogenic effects.

If observed effects are caused by toxicity, dose-response testing should be conducted providing reliable ecotoxicological endpoints (EC_{50}). The Canadian Guidelines provide the option of multi-concentration tests. These endpoints can be integrated in standard risk assessments similar to chemical pesticides including the use of established safety factors. Exploring the origin of effects observed in control groups receiving an attenuated treatment (microbe-free or nonviable microbe comprising material from the culture system used for propagation) might be helpful, as adverse effects caused by this treatment are not due to pathogenicity.

According to the US EPA guidelines OCSPP 885.4300, one concentration level equal to no less than the maximum label rate shall be tested. The phrase "maximum label rate" means the amount of active ingredient in the recommended quantity of carrier, such as water to be used per land area or applied directly to the surface of a 15 cm or 6 inch column of water. According to Canadian guidance, a MHC for soil of 10^6 microbial units/g soil (dry wt) or 1000 times the expected microbial concentration in soil within the terrestrial environment is defined. A subsequent risk calculation requiring an adequate exposure assessment is dispensable provided that no adverse effects are observed.

In case of pathogenic effects, these observations have to be considered and classified in context of the overall-knowledge about the mBCA (physiological and ecological host range, mode of action, life-cycle and biology of the mBCA, environmental conditions for survival, germination and infection).

Waiver options

A waiver can be submitted:

- If exposure of terrestrial plants is negligible or minimal.

- In case of mBCAs with no intended use as a herbicide, if database searches find no reports of detrimental impacts to plants by the considered microorganisms and relative species within the

same genus in connection with sufficient information about mode of action, biology, life cycle and environmental conditions for survival and reproduction.

4.3.5 Earthworms

General aspects

The issue of possible adverse effects (toxicity, infectivity and pathogenicity) to earthworms has to be addressed according to point 8.5 in part B in the annex to Regulation (EU) No 544/2011 when applying for the registration of mBCAs.

US EPA has no data requirement assessing the risk to earthworms. REBECA[3] questions the reasonability of earthworm tests and states "no earthworm pathogens have been reported". Moreover, it is argued that earthworms should be adapted to soilborne bacteria and fungi as these are often similar to mBCAs applied for registration. Indeed, searching for microbial earthworm pathogens is difficult compared with, *e.g.*, plant or insect pathogens, as there are free databases for diseases of plant or insects but not for annelids.

Until now, only few studies indicated pathogenic effects to earthworms (see below).

For example, Smirnoff and Heimpel (1961) reported pathogenic effects of *B. thuringiensis* subsp. *thuringiensis* (Thuricide® 30B) in a prolonged study with *Lumbricus terrestris*. However, further analysis revealed that observed lethal effects were not attributed to the mBCA. As explained in the review by Addison J.A. (1993) reported effects were caused by the presence of diatomaceous earth used as carrier in the formulated product.

Detrimental impacts of *Bt*-formulations on earthworms and other non-target soil organisms have also been reported in studies by Addison and Holmes (1995, 1996). The authors examined effects of *Bt* subsp. *kurstaki* on a forest earthworm (*Dendrobaena octaedra*) and found no effect of unformulated and aqueous *Btk* at 1000 times the field concentration, however an oil formulation of *Btk* reduced survival, growth and reproduction (Addison and Holmes, 1996).

An earthworm study with *B. subtilis* QST 713 (Serenade® WP) submitted during the European process of Annex I inclusion showed sublethal effects such as lethargy and reduced reaction to mechanical stimuli. Histopathological analysis revealed bacterial colonisation in body tissues. However, it should be noted that these observations occurred at concentrations that would not be expected under field conditions.

Another example of pathogenic effects to earthworms was published by Vakili (1993). The author described the isolation of the soilborne fungus *Exophiala jeanselmei* from cocoons of the earthworm species *Eisenia foetida* infected by the fungus. As several attempts to isolate the fungus from the used soil were not successful, it was concluded that the fungus was carried within the body of the adult earthworms and disseminated to their cocoons. Adult earthworms were not described as negatively affected. Deleterious effects were observed only in the reproduction process. The examples above indicate that standard short-term 14-day earthworm studies focusing on detecting lethal or sublethal effects (weight loss) are probably not suitable to prove the absence of infectivity or pathogenicity at least by visual observation.

[3] **REBECA** is an EU policy support action to review possible risks of biocontrol agents, compare regulations in the EU and the US and to propose alternative, less bureaucratic and more efficient regulation procedures maintaining the same level of safety for human health and the environment but accelerating market access and lowering registration costs.

Earthworms obviously cope with soilborne microorganisms without being infected or negatively affected. This is due to the long time of evolutionary earthworm-pathogen co-existence, during which earthworms have developed. Earthworms developed an immune system being native, mainly non-specific, non-anticipatory and non-clonal. Although there was evidence of specific memory in immune response of invertebrates under certain conditions, this is limited to the recognition of PAMPs (pathogen-associated molecular patterns) being present in many microorganisms mediated by pattern recognition receptors like Toll-like receptors (Rowley and Powell, 2007). Therefore, evidence suggests that the immune strategy of earthworms is similar to various microorganisms.

Available test guidelines

1) Suitable guidelines determining effects of mBCAs to non-arthropod invertebrates such as earthworms are considered in the Canadian registration procedure under PMRA Data Code: M9.6 Non-arthropod invertebrates (PMRA, 2001).

2) The following methodology is recommended according to Section 13 of the Canadian guidance document (Environment Canada, 2004):

   - *Test species*: *Eisenia andrei* (also referred to as *Eisenia foetida andrei*) or the closely related species *Eisenia fetida.*

   - *Testing for infectivity:* Infectivity is examined by measuring the concentration of microbial substance in whole-organism homogenate of earthworms from each treatment during and/or at the end of the test.

   - *Route of exposure:* The route of exposure is via soil mixed with the test substance or via food.

   - *Test concentration* It is possible to use only one concentration (i.e., MHC) in a single concentration test or a minimum of seven concentrations including the MHC. The MHC used for soil mixture is defined as 10^6 microbial units/ g soil (dry wt), or 1000 times the expected microbial concentration in soil within the terrestrial environment. The definition of the MHC used in food mixture is 100 times that in the maximum concentration of microorganisms specified by the notifier for the final tank mix of a microbial product.

   - *Control:* Negative (untreated) as well as positive controls have to be included. The use of a non-infectious control is strongly recommended. A sterile filtrate control is optional but also recommended.

   - *Observations and biological endpoints:* The total number of live adult worms on Days 0 and 28, number of live juvenile worms on Day 56, obvious pathological symptoms (*e.g.* open wounds) or distinct behavioural abnormalities (*e.g.* lethargy) have to be recorded. Biological endpoints are determined for the total number of surviving adult worms on Day 28, total dry weight and number of surviving juvenile worms on Day 56, number of surviving adult worms showing atypical appearance and/or behaviour on Day 28 as well as on Day 56.

   - *Test duration*: Effects on survival, reproduction and growth are to be detected within a timeframe of 56 days.

   - *Criteria of validity:* Tests are considered invalid if mean 28-day survival of adults in negative control soil < 90 %, if mean reproduction rate for adults in negative control soil < 3 live

juvenile/adult and if mean dry weight of individual live juveniles in negative control soil at test end < 2 mg.

General remarks regarding the risk assessment for below ground NTOs

The estimation of the PIECsoil might give an overall idea of the exposure of below ground NTO (earthworms, microorganisms, other NTO). Care has to be taken with the use of the NOEC and LC_{50} values which are also calculated with a substantial uncertainty. For most mBCAs, a qualitative risk assessment based on an overall view of all available information is the most relevant and often the only possible evaluation. When using OCSPP-tests (first Tier) endpoints are usually based on MHC and subsequently LC_{50} values if effects are observed in an initial stage. LC_{50} values are generally not suitable for assessing risks of pathogenicity since infection/pathogenicity do not necessarily occur in a dose-response manner. Likewise, an attempt to derive a specific pathogenic threshold level is not deemed feasible.

The European regulation demands data concerning earthworms and soil-microorganisms that are not required in the US. However, there are Canadian test guidelines addressing the determination of adverse effects of mBCAs to earthworms as well as springtails. Therefore, studies might be conducted in accordance with these guidelines.

Risk assessment

A risk calculation analogous to chemicals is generally regarded as less feasible for the risk assessments of mBCAs because dose-response relationships are rarely observed in cases of pathogenic effects.

If observed effects are caused by toxicity, dose-response testing should be conducted providing reliable ecotoxicological endpoints ($LC_{50,}$ LR_{50}, NOEC). The Canadian Guidelines provide the option of multi-concentration tests. These endpoints can be integrated in standard risk assessments similar to chemical pesticides including the use of established safety factors. Exploring the origin of effects observed in the control groups receiving an attenuated treatment (microbe-free or nonviable microbe comprising material from the culture system used for propagation) might be helpful, as adverse effects caused by this treatment are not due to pathogenicity.

According to Canadian guidelines, a MHC for soil of 10^6 microbial units/g soil (dry wt) or 1000 times the expected microbial concentration in soil and 100 times the maximum concentration of microorganisms specified by the notifier for the final tank mix of a microbial product for food mixture is defined. A subsequent risk calculation requiring an adequate exposure assessment is dispensable provided that no adverse effects are observed.

In instances of pathogenic effects, these observations have to be considered and classified in context of the overall-knowledge about the mBCA (physiological and ecological host range, mode of action, life-cycle and biology of the mBCA, environmental conditions for survival, germination and infection).

Waiver options

A waiver can be submitted:

- If exposure of earthworms is negligible or minimal.

- If in-depth knowledge is available on the mode of action, biology, life cycle and environmental conditions for survival and reproduction of the mBCA excluding any risk to earthworms (*e.g.* quality of information known for baculoviruses).

4.3.6 Non-target soil microorganisms

The US EPA BPPD microbiologists do not support testing for effects of microbial pesticides on microorganisms for the following reasons:

- There may be effects from almost anything added to the soil, but there is no valid way to interpret any results one might obtain from testing.

- The relative risk from adding microorganisms to the soil microbial community is minimal. Soil microflora varies immensely spatially and temporally. The natural population has adapted to their particular environmental niches, and has evolved many defense mechanisms to allow their survival in those niches.

- Soil microflora is very resilient, *e.g.* even when the microbial populations are decimated by methyl bromide, the natural soil populations rebound quickly.

US EPA notes that it is a valid area for research, but is not a very significant risk issue in the big picture.

In the EU dossiers for the inclusion of mBCAs in Annex I, nitrification and respiration tests were submitted for *Beauveria bassiana* strain ATCC 74040, *Trichoderma asperellum* strain ICC012 (formerly *Trichoderma harzianum* Rifai), *Trichoderma gamsii* strain ICC080 (formerly *Trichoderma viride* strain ICC080), *Bacillus thuringiensis* subsp. *aizawai* GC-91, *B. thuringiensis* subsp. *tenebrionis* NB-176, *B. thuringiensis* subsp. *kurstaki* ABTS-351, PB54, SA-11, SA-12 and EG2348. These functional tests did not show any adverse effects. This supports the assumption of the resilience of soil-microflora especially concerning issues of functionality of the microbial community in soil. Therefore, standard nitrification and respiration tests according OECD Guidelines 216/217 are less suitable to fullfil this data requirement. Besides, it is worth mentioning that such studies according to OECD 216/217 are designed for chemicals and are not validated for mBCAs as test substance.

In conclusion, the relevance of carbon mineralization and nitrogen transformation tests seems to be low as indicated in previous BPSG seminar presentations. However, impacts on microbial community structures (Pérez-Piqueres *et al.*, 2006; Edel-Hermann *et al.*, 2009) or on symbiotic activity of arbuscular mycorrhizal fungi may be of relevance in some cases. Studies on these two topics were submitted in the EU dossiers for the inclusion of *Trichoderma atroviride* I-1237 and *Trichoderma asperellum* strain T34 in Annex I of EU Directive 91/414/EEC (now Regulation (EC) No 1107/2009).

For the EU review process of other mBCAs, literature information, waivers or statements were submitted. Occasionally, inhibition of microorganisms by an mBCA (e.g. *B. thuringiensis* subsp. *tenebrionis* NB-176) has been studied using culture techniques (*e.g.* agar plates), microbial biomass or

enzyme activity (*e.g.* dehydrogenase). In fact, not one particular test is prescribed and applicants can choose from a wider range of techniques and also, the non-target group to be tested needs to be chosen (*e.g.* fungi, bacteria, actinomycetes or protozoa).

For the sake of increasing knowledge on the non-target effects of mBCAs on non-target soil microorganisms, a desk study by Scheepmaker and Van de Kassteele (2011) was initiated. In this study, the effects of chemical pesticides and mBCAs (*Azospirillum, Burkholderia, Clonostachys, Pseudomonas, Streptomyces, Trichoderma, Bacillus, Beauveria, Metarhizium*) on non-target microorganisms were compared. All data derived from published and peer-reviewed studies. Investigated non-target groups were bacteria, fungi, actinomycetes and protozoa. It was shown that the effects of mBCAs are followed by recovery within 100 days. Initial effects caused by mBCAs can be either negative or positive. Application of antagonists, the fungal antagonist in specific, results in initial increases of numbers of bacteria. Most likely, the antagonists are a rich nutrient source for the resident bacterial population, resulting in rapid increases of their numbers. The fact that initial effects are short term is in agreement with the current EU approach that recovery should be observed within an ecological relevant period.

In those cases where actual studies are required to fulfill the data requirement for non-target soil microorganisms, it is advised to test the most sensitive mBCA/non-target combinations.

This evaluation provides a general picture of expected effect of mBCAs on various groups of soil microorganisms. Moreover, these analyses can be the basis for a critical discussion of the usefulness of such information in risk assessments of new mBCAs.

A similar meta regression study is currently performed on the effects of chemical control agents and microbial biocontrol agents on soil enzyme activities (Scheepmaker *et al.*, in prep.).

Waiver options

A waiver can be submitted:

- If exposure of soil microorganisms is negligible or minimal.
- If a search of published scientific literature finds that effects on the soil microflora are minor and transient.

4.4 Aquatic NTOs

If significant exposure of aquatic organisms can be expected following application, toxicity/pathogenicity tests for fish, aquatic invertebrates and algae are required, unless it can be justified that an assessment of effects on NTOs can be performed with information already available in the open scientific literature. Testing of aquatic plants may be useful if the mBCA is taxonomically related to a known plant pathogen.

Since an mBCA that reaches the water surface may tend to precipitate to the sediment it may be useful to require studies on the effects on sediment-dwelling organisms such as epibenthic chironomid larvae or endobenthic annelids. However, it should be emphasized that the choice of test organisms should be verified based on the information on the biological properties (mode of action, host range) as this step is crucial for the assessment of impact on non-target species.

4.4.1 Fish

General aspects

The possibility of adverse effects to fish has to be addressed adequately if exposure of surface water is expected following application of the mBCA. This requirement is required by the EU as well as the US and Canada.

Available test guidelines

1) US EPA provides a test guideline on freshwater fish testing in Tier I (OCSPP 885.4200).

   - *Test species:* In cases of indirect application such as spray drift, one test species is required, preferably rainbow trout. In cases of direct applications to water, two test species are required, preferably rainbow trout and bluegill sunfish. Furthermore, the use of young fish is recommended.

   - *Route of exposure:* Test organisms are to be exposed by two routes, first via mBCA suspended directly into the water and second via mBCA mixed with food (at least 100 times the calculated cell density/mL in a 6–inch (15 cm) layer of water immediately following a direct application to a 6–inch (15 cm) layer of water).

   - *Test concentrations:* Tests are to be conducted with a MHC being 10^6 units/mL or 1000 times the expected microbial concentration in the aqueous environment.

   - *Control:* A negative, non dosed control group should be run concurrently with the test groups. A control group in which the fish are exposed to sterile filtrate from production cultures should be performed concurrently with the test groups.

   - *Test duration:* The test duration is greater than or equal to 30 days.

2) Environment Canada recommends a test method mainly adapted from the OECD guideline No. 215 ("Fish, Juvenile Growth Test), with additional guidance from US EPA test guidelines OCSPP 885.4200 and the ASTM "Standard guide for conducting Bioconcentration tests with Fishes and Saltwater Bivalve Molluscs". This approach is consistent with conditions demanded in the US EPA guidelines.

   - *Test species*: Test species are comparable to the US EPA guidelines.

   - *Type of study:* Studies are to be conducted as static renewal tests.

   - *Testing for infectivity:* Tests for infectivity are optional.

   - *Route of exposure*: route of exposure is comparable to the US EPA guidelines.

   - *Test concentration:* definitions of the MHC are comparable to the US EPA guidelines.

   - *Control:* Additionally to controls required in the US EPA guidelines, the use of a non-infectious control is strongly recommended.

- *Observations and biological endpoints:* Besides observations of fish survival, appearance and behaviour, necropsy upon death of each fish during test and at test end, histological investigations of selected tissues and organs have to be conducted.

- *Test duration:* The test duration is a minimum of 28 days.

Advantages of the Canadian test guidelines are the inclusion of the multi-concentration testing approach, criteria for test validity and specific recommendations about experimental conditions.

Risk assessment

Risk calculation analogous to chemicals is generally regarded as less feasible for the risk assessments of mBCAs because dose-response relationships are rarely observed in cases of pathogenic effects. If observed effects are caused by toxicity, dose-response testing should be conducted providing reliable ecotoxicological endpoints (LC_{50}, EC_{50}, NOEC/LOEC). The Canadian guidelines provide the option of multi-concentration tests. These endpoints can be integrated in standard risk assessments similar to chemical pesticides including the use of established safety factors. Exploring the origin of effects observed in control groups receiving an attenuated treatment (microbe-free or non-viable microbe comprising material from the culture system used for propagation) might be helpful, as adverse effects caused by this treatment cannot be attributed to pathogenicity.

According to US EPA Test guidelines OCSPP 885.4200 and the Canadian guidelines, a MHC for water of 10^6 microbial units/mL water or 1000 times the expected microbial concentration in water bodies is defined. The MHC for exposure via food is at least 100 times the calculated cell density per millilitre in a 6–inch (15 cm) layer of water immediately following a direct application to a 6–inch (15 cm) layer of water. A subsequent risk calculation requiring an adequate exposure assessment is dispensable provided that no adverse effects are observed.

In cases of pathogenic effects, these observations have to be considered and classified in context of the overall-knowledge about the mBCA (physiological and ecological host range, mode of action, life-cycle and biology of the mBCA, environmental conditions for survival, germination, and infection).

Waiver options

A waiver can be submitted:

- If exposure of fish is negligible or minimal.

- If a microorganism is not able to survive in surface water and sediment.

- If database searches find no reports of detrimental impacts to fish caused by the considered microorganisms and relative species within the same subfamily or genus in connection with sufficient information about mode of action, biology, life cycle and environmental conditions for survival and reproduction.

4.4.2 Aquatic invertebrates

General aspects

The possibility of adverse effects to aquatic invertebrates has to be addressed adequately if exposure of surface water is expected following the intended application of the mBCA. This requirement is required by the EU as well as the US and Canada.

Available test guidelines

1) US EPA guidelines OCSPP 885.4240

- *Test species:* One species of benthic invertebrates should be tested in cases of indirect exposure of surface water, *e.g.* spray drift of terrestrial spray applications. If the mBCA is intended to be applied directly into water bodies, a second planktonic invertebrate has to be tested. The selected test species should be preferably closely-related to the target host or test organisms should be chosen likely to prey upon or scavenge the diseased target host organisms (in case of entomopathogenic mBCAs). Larval life stages are preferred.

- *Test concentration:* The test substance is applied as a MHC being 10^6 units/mL or 1000 times the expected microbial concentration in the aqueous environment and is suspended directly into the test water.

- *Control:* A negative non-dosed control group as well as a control group exposed to sterile filtrate from production cultures is to be performed concurrently.

- *Observations and biological endpoints:* Test organisms are examined for any behavioural, pathogenic, or toxic effects and especially for infection or any microorganism-related effects periodically throughout the study and at test termination.

- *Test duration:* The test duration is 21 days.

2) Environment Canada

Two guidelines are recommended for testing aquatic freshwater invertebrates, one using the freshwater cladoceran *Daphnia magna,* the other using the larvae of freshwater midges *Chironomus tentans* or *Chironomus riparius*. The study guidelines for *D. magna* are an appropriately adapted version of the OECD guidelines 211 "*Daphnia magna* reproduction test:

- *Test concentration*: Test substances can be applied as an MHC being similar to MHC defined in the US EPA guidelines or in a minimum of five concentrations including the MHC.

- *Control:* Beside negative control and sterile filtrate control (optional) groups, a non-infectious control is strongly recommended.

- *Observations and biological endpoints:* Survival of parental daphnid and success of reproduction is required whereas testing for infection by measurements of the microbial concentration in whole-body homogenate is optional.

- *Test duration:* The test duration is 21 days.

Advantages of the OECD 211-adapted test guidelines are the presence of test validity criteria and well established test conditions. However, it was noted that the taxonomic relatedness to the host species is not taken into account. Thus possible pathogenic effects to aquatic invertebrates remain unrecognised when using solely *D. magna* as test species. Searching appropriate databases (see below) for potentially negative impacts of mBCAs and species being closely related to the mBCA might help to assess whether pathogenic/toxic effects to other aquatic invertebrates are probably overlooked.

Tests with the freshwater sediment invertebrate *Chironomus tentans* or *C. riparius* may be required when mBCAs precipitate quickly into sediment and can probably persist in the sediment for a significant amount of time (preconditions still have to be discussed). Environment Canada provides test guidelines adapted from EC test guidelines designed for chemical substances "Test for Survival and Growth in Sediment Using the Larvae of Freshwater Midges (*Chironomus tentans* or *Chironomus riparius)*".

- Test organisms are third instar *C. tentans* and first instar *C. riparius*.

- Test substances have to be mixed in both freshwater and sediment.

- A single-concentration test using the MHC defined as above or a multi-concentration test with a minimum of five concentrations including the MHC is possible.

- A negative control, a positive (chemical) control must be determined whereas the concurrent performance of a non-infectious control is strongly recommended and a sterile filtrate control is optional.

- Observations include numbers of midge larvae on sediment surface, and assessing their behaviour, appearance and survival. The mean dry weight has to be measured. Testing for infection by measuring the microbial concentration in whole-body homogenate is optional.

- The test guidelines provide specific experimental conditions and validity criteria.

Risk assessment

A risk calculation analogous to chemicals is generally regarded as less feasible for the risk assessments of mBCAs because dose-response relationships are rarely observed in cases of pathogenic effects. If observed effects are caused by toxicity, dose-response testing should be conducted providing reliable ecotoxicological endpoints (LC_{50}, EC_{50}, NOEC/LOEC). The Canadian guidelines provide the option of multi-concentration tests. These endpoints can be integrated in standard risk assessments similar to chemical pesticides including the use of established safety factors. Exploring the origin of effects observed in control groups receiving an attenuated treatment (microbe-free or non-viable microbe comprising material from the culture system used for propagation) might be helpful, as adverse effects caused by this treatment cannot be attributed to pathogenicity. According to US EPA Test guidelines

OCSPP 885.4240 and the Canadian guidelines, a MHC for water of 10^6 microbial units/mL water or 1000 times the expected microbial concentration in water bodies is defined. A subsequent risk calculation requiring an adequate exposure assessment is dispensable provided that no adverse effects are observed. In instances of pathogenic effects, these observations have to be considered and classified in context of the overall-knowledge about the mBCA (physiological and ecological host range, mode of action, life-cycle and biology of the mBCA, environmental conditions for survival, germination, and infection).

Waiver options

A waiver can be submitted:

- If exposure of aquatic invertebrates is negligible or minimal.
- In case of non-entomopathogenic mBCA, if database searches find no reports of detrimental impacts of the considered microorganisms and relative species within the same subfamily or genus to aquatic as well as terrestrial (soil- or leaf-dwelling invertebrates) in connection with sufficient information about mode of action, biology, life cycle and environmental conditions for survival and reproduction.
- If a microorganism is not able to survive in surface water and sediment.

4.4.3 Aquatic plants (including algae)

General aspects

The possibility of adverse effects to aquatic plants has to be addressed adequately if exposure of surface water is expected following the intended application of the mBCA. This requirement is required by the EU as well as the US and Canada.

Available test guidelines

1) Commission Regulation (EU) No 544/2011

According to this regulation, information on effects on algal growth, growth rate and capacity to recover must be reported and effects on aquatic plants other than algae must be reported for mBCAs by the applicant. No definite test species is defined. However, standard test species for testing chemicals according OECD test guidelines 201/221 are green algae *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*), *Desmodesmus subspicatus* (formerly known as *Selenastrum subspicatus*), Cyanobacteria *Anabaena flos-aquae* and S*ynechococcus leopoliensis* and the diatom *Navicula pelliculosa*. The gibbous duckweed, *Lemna gibba,* or the common duckweed, *L. minor,* are used as a standard test species representative for macrophytic aquatic plants. In rare cases where concern exists that the fast-growing *Lemna* species may underestimate risks, other aquatic macrophytes such as the rooted dicotyle macrophyte *Myriophyllum aquaticum* (parrot feather watermilfoil) is commonly used as test species (optionally conducted with or without sediment). However, an internationally recognised test method for the latter species is currently lacking.

2) US EPA test guidelines OCSPP 885.4300 "Nontarget Plant Studies Tier I"

Within these guidelines, only a short paragraph refers to aquatic plants and it addresses the issue of appropriate test species. Accordingly, the green algae *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*), the blue-green alga *Anabaena flos-aquae* and the duckweed *L. gibba* are to be examined. Furthermore, the marine diatom *Skeletonema costatum* (for application near coasts) and another freshwater diatom (e.g. *Navicula pelliculosa*) must be tested. According to the US guidelines, testing is required for aquatic uses or in cases of expected disseminations of the mBCA to aquatic ecosystems. Another precondition for test requirements is the potential of the mBCA to survive in natural water bodies. No test-specific biological methods are provided for these species in this or other Series 885 test guidelines.

3) Environment Canada test guidelines

These guidelines prefer the aquatic duckweed *Lemna* sp. as test species. In the Canadian guidance document, an adapted version of Environment Canada duckweed growth inhibition test for chemicals. *Lemna minor* as test organism is to be exposed to the mBCA for 7 days with a renewal of each test concentration at least twice, on Days 3 and 5 of the test. Test substances can be applied as an MHC (10^6 units/mL or 1000 times the expected microbial concentration in the aqueous environment) or in a minimum of five concentrations including the MHC. Growth rates are calculated on the basis of measured number of fronds and dry weight at test start and test end. Plant appearance has to be observed at start and end of the test. Testing for infectivity based on measured concentrations of the mBCA in whole-body homogenates of *L. minor* is optional. Beside negative control and sterile filtrate control (optional) groups, a non-infectious control is strongly recommended. Detailed information on test conditions is given as well as criteria for validity. One disadvantage of this method is the shortness of test duration as possible pathogenic effects might not manifest during 7 days of exposure. Extending the duration of the test might be a logical solution but increasing the exposure period requires a change of validity criteria. Alternatively, an adequately adapted version of the OECD test guidelines No. 221 may also be accepted according to the Canadian guidance document. With a limited test duration of only 7 days, these guidelines have the same shortcoming as the Canadian guidelines and possibly require the same determination of new validity criteria.

In contrast to European data requirements, studies assessing the effects of a mBCA to algal growth are usually not required in the US and Canada. US EPA Biopesticide Division staff scientists had commented on February 8, 2011 as follows: "Although the US guidelines 885.4300 mention that aquatic algae may be studied, we are not aware of any case where we have asked for algae to be tested. It would be very unusual to find a microbial pesticide that could present a persistent effect on a beneficial alga."

As stated in the Canadian guidance document, the adoption of established standard tests for algae, *e.g.*, according to OECD Test guidelines No. 201, is difficult since algal studies are not compatible with static renewal test methods. When using relatively high maximum hazard doses/concentrations, static renewals are often required to maintain water quality and constant exposure. Lowering the initial test concentration may circumvent this problem but this concentration might be regarded as insufficient when examining possible pathogenic effects to algae.

Risk assessment

A risk calculation analogous to chemicals is generally regarded as less feasible for the risk assessment of mBCAs because dose-response relationships are rarely observed in cases of pathogenic effects. If observed effects are caused by toxicity, dose-response testing should be conducted providing reliable ecotoxicological endpoints (LC_{50}, EC_{50}, NOEC/LOEC). The Canadian guidelines provide the option of multi-concentration tests. These endpoints can be integrated in standard risk assessments similar to chemical pesticides including the use of established safety factors. Exploring the origin of effects observed in control groups receiving an attenuated treatment (microbe-free or non-viable microbe comprising material from the culture system used for propagation) might be helpful, as adverse effects caused by this treatment cannot be not due to pathogenicity. According to the Canadian guidelines, a MHC for water of 10^6 microbial units/mL water or 1000 times the expected microbial concentration in water bodies is defined. A subsequent risk calculation requiring an adequate exposure assessment is dispensable provided that no adverse effects are observed. In instances of pathogenic effects, these observations have to be considered and classified in context of overall-knowledge about the mBCA (physiological and ecological host range, mode of action, life-cycle and biology of the mBCA, environmental conditions for survival, germination and infection).

Waiver options

A waiver can be submitted:

- If exposure of aquatic plants is negligible or minimal.
- In case of mBCAs with no intended herbicidal uses, if database searches find no reports of detrimental impacts to plants by the considered microorganisms and relative species within the same genus in connection with sufficient information about mode of action, biology, life cycle and environmental conditions for survival and reproduction.

5. Refinement options (BOX 6 of decision scheme)

In case the risks are deemed to be too high, the risks can be refined by performing Tier 2 or even Tier 3 experiments. This possibility for refinement is common in the risk assessment of chemical pesticides. For mBCAs, this option will hardly ever be used. US EPA Biopesticides Division staff scientists had commented on January 29, 2010, that they have never needed microbial pesticide NTO studies that would give an LD_{50}. Tier 2 level data that are required only if toxic or pathogenic effects are seen in the Tier I tests have also not been required to date. In contrast to the situation in the US, requests of higher Tier (semi-field) tests may have occurred more frequently in the EU registration process, for instance a bumble bee study with an mBCA under greenhouse conditions was submitted in the context of national approval in Germany.

6. Mitigation options (BOX 7 of decision scheme)

Another option to reduce risks is the possibility of mitigation. Well-known possibilities used for chemical pesticides are

- Reducing application rates. This option seems to be impracticable since the mode of action of many mBCAs can be pathogenic and reduced application rates may come into conflict with the efficacy of a product. No dose-effect correlation is available with such mBCAs and thus, reduction of applied concentrations is not useful.

- Using drift reducing nozzles. This option can be used when risks are expected to be caused by spray drift to aquatic organisms or to NTO in adjoining off-crop areas.

- Establishing buffer zones along areas of surface water or off-crop areas to reduce exposure of surface water to spray drift.

- Rejecting an intended use with unacceptable risks. Although this option is actually not considered a real mitigation option, this step will eventually be taken when no other options are available.

- Preventing applications to flowering crops to avoid exposure of pollinators. This is a specific measure to avoid risks for bees and other wild pollinators.

7. Issues to be solved in the near future

In order to harmonise risk assessment procedures among different regulatory agencies, the following issues that need to be solved in the near future, are highlighted:

- Assessment of fungal metabolites. A publication is in preparation by Butt *et al.* (in prep.) that categorises metabolites/toxins and their effects on NTOs from the literature. Risk strategies will be proposed.

- Other options to improve the qualitative risk assessment of mBCAs, such as better insight into fate and behaviour of mBCAs.

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[4] International Biocontrol Manufacturers' Association

[5] European Commission Joint Research Centre, Institute for Health and Consumer Protection, Chemical Assessment and Testing Unit

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APPENDIX: SUGGESTIONS FOR RELEVANT DATABASES

Non-target organisms (including birds and mammals)

German Institute of Medical Documentation and Information (DIMDI)

http://www.dimdi.de/static/en/index.html (last accessed, April 27, 2011)

Terrestrial plants

University of Bonn provides a Plant Pathology Internet Guidebook (PPIGB)

http://www.pk.uni-bonn.de/ppigb/menu.htm (last accessed, April 27, 2011)

Aquatic organisms

http://www.diplectanum.dsl.pipex.com/purls/host.htm (last accessed, April 27, 2011)

http://www.fao.org/DOCREP/003/X9199E/X9199E03.htm (last accessed, April 27, 2011)

Terrestrial invertebrates

http://cricket.inhs.uiuc.edu/edwipweb/edwipabout.htm (last accessed, April 27, 2011)

http://arthropodenkrankheiten.jki.bund.de/index_e.php (last accessed, April 27, 2011)