GUIDELINES

Framework for Assessing the Environmental Safety of Microbial Pesticides

CropLife International's Guidelines, established January 2018

Contents

Background

Microbial pesticide products are a sub-set of the much larger group of 'Biologicals' which are all derived from natural occurring materials or organisms. In the United States the following specific definition is given for microbial pesticides in 40 CFR 158.2100.

Microbial pesticides are defined as a microbial agent intended for preventing, destroying, repelling, or mitigating any pest, or intended for use as a plant regulator, defoliant, or desiccant, that:

1. *Is a eucaryotic microorganism including, but not limited to, protozoa, algae, and fungi;*

2. *Is a procaryotic microorganism, including, but not limited to, Eubacteria and Archaebacteria; or*

3. *Is a parasitically replicating microscopic element, including, but not limited to, viruses.*

Definitions of Biologicals and how they are subdivided vary across the globe; however when assessing the safety of a Biological the specific regulatory classification in any particular country/region is of limited relevance.

Purpose

This document is intended to provide a framework for how to assess the environmental safety of microbial pesticides. This is intended to aid company internal decision-making regarding safety but does not assess what is required to gain regulatory approval in any particular country. Typical situations where the framework may be applied can be a due diligence exercise for a 3rd party product/AI or a release to first sales decision for a 3rd party product/AI based on often limited available data. Further, it may be useful during early stage development projects.

Scope

The scope of this document covers microbial pesticide products as defined above and for clarity covers:

- Living and non-living organisms (e.g. via heat inactivation)
- Genetically modified microbial products but not RNAi
- Extracts of microbial origin

Specific considerations relating to the manufacturing specification and/or Product Chemistry requirements are out of scope of this document; however this area is critical in underpinning the safety assessment of these products and some general considerations are discussed.

Although within the scope of this guidance document, non-living microorganisms and extracts do not fall under the scope of the legislation for microbial pesticide in all jurisdictions; extracts may be regulated as biochemicals.
Pathogenicity studies as discussed in the following are obviously of relevance for living organisms only.

Regulatory requirements for assessing environmental safety for microbial pesticides

Criteria for selecting environmental safety data must be broad to cover all microbial products. Furthermore, regulators seem flexible in their approach to data requirements. If, for instance, an acceptable environmental risk is shown using existing data and literature, regulators are likely to waive study requirements. Although there are some differences between the regulations in different countries/regions, the studies in Table 1 are usually required for the registration of microbial products. In some circumstances Industry may position that less data is needed to demonstrate safety than is required by a country / region whereas in others more data than required may be necessary to fully understand the environmental safety profile. The extent of the required ecotoxicity testing generally depends on the use pattern of the product and thus on exposure of potential non-target organisms.

The following table lists studies that may be considered to help determine the safety of a microbial product.

Description	Test Material	Guideline Reference
Avian oral toxicity	TGAI*	OPPTS 885.4050
Avian Inhalation toxicity/ pathogenicity*	TGAI**	OPPTS 885.4100
Freshwater fish toxicity testing is required to be conducted for 30 days exposure although histopathological and blood work-up portion of assay need not to be conducted (if pathogenicity is not suspected)	TGAI	OPPTS 885.4200
Test with the *Cladoceran* (*Daphnia magna*)	TGAI	OPPTS 885.4240
Estuarine and marine animal testing, Tier I*	TGAI	OPPTS 885.4280
Algal toxicity testing *	TGAI	OECD 201 or OCSPP 850.4500
Aquatic macrophyte testing*	TGAI	OECD 221 or OCSPP 850.4400
Honey bee testing	TGAI or EP	OPPTS 885.4380
Non-target arthropods other than bees	TGAI or EP	OPPTS 885.4340 or Escort 2 depending upon region

Description	Test Material	Guideline Reference
Earthworms	TGAI	OECD 207
Soil micro-organisms (N-, C-cycling)	TGAI	OECD 216/217

TGAI – Technical grade active ingredient

EP – End-use product

*) typically waived or conducted only under very specific circumstances

**) TGAI may be modified to facilitate administration as some types of biologicals cannot be administered as TGAI

Although the above data may be required, consideration needs to be given to the nature and selectivity of the microbial towards non-target organisms, and the available literature, to decide if it is appropriate to conduct the studies or if data waivers will suffice. Although formal waiver system do not always exist, there is usually an opportunity to present a reasoned case as to why studies are not required.

Micro-organisms are regulated at the strain level in all jurisdictions where regulations exist. Each "new" variety, subspecies, or strain of an already registered microbial pest control agent must be evaluated. Strains may be assessed together, if they are "similar" although this will need justification. Non-indigenous micro-organisms may be subject to additional data requirements. All regulatory systems follow a case-by case approach in the evaluation of micro-organisms for the use in microbial plant protection products.

Safety standards and evaluation

In assessing the safety of any microbial product, consideration needs to be given to the following:

- What is the nature of the microbial product (whole organism, endospore, extract etc.)?
- What is the host range?
- Is it a native or non-native species?
- What are the natural background levels in the environment?
- Can a non-native species become native?
- What is the proposed use pattern?
- Is it genetically stable?
- What is the potential for genetic transfer?
- How is it mobile in the environment?
- How is it produced?

For the majority of those points data should be available from the open literature although the relevance and quality of the data need consideration. In addition to literature data there may be evidence of existing natural exposure or indeed the microbial product may be registered in other

territories, or for other uses, and this should all be considered as part of any initial safety assessment. It should also be noted that microbial products are chosen to control living organisms (insects, fungi, plants). The target organisms may be related to non-target organisms (e.g. *Apis mellifera*), for which protection goals exist. Careful consideration is needed with respect to target organism(s) and the selectivity of the microbial product.

1. Identity and quality control

The basis for a safety assessment of a microorganism is the proper identification, taxonomic and genetic characterisation and an assessment of the potential for secondary metabolite formation. A detailed review of the available scientific literature usually provides this information. An appropriate set of screening studies and/or guideline studies as outlined below will further allow to develop the needed knowledge for a proper assess the safety of the organism and the respective pesticidal product.

Most microbes used in products are produced using a live fermentation process. This introduces the potential for 'contamination' of the product with extraneous materials – this could be different strains of the intended microbe or could be other micro-organisms, pathogens or other secondary metabolites/toxins.

It is important at the outset of any evaluation of a microbial product, based on the identity of the intended microbe, to introduce adequate quality control during the production process i.e. ensuring production of the intended product. From a safety perspective, it is necessary to demonstrate an axenic culture of the intended organism and lack of unintended contamination. Therefore, quality control measures must be specific to identify the intended microbe in the presence of revertants/mutants and contaminants that may have been formed or introduced during the replication/manufacturing process (e.g. refer to OPPTS 885.1500). Also, if the culture conditions are permissive of the growth of human or animal pathogens, e.g. *Enterobacteriaceae*, appropriate quality control measures need to be put in place which can go as far as testing of each production batch for their presence and suitable methodology proposed for testing and/or eliminating them (e.g. refer to OPPTS 885.1300).

Any experimental data and evaluations also need to be appropriate to the species being produced. Basic information regarding the identity and characteristics of the microbial product can be useful in predicting effects against non-target organisms. With respect to, for example, insecticidal microbial products, attention should be given to the selectivity of the product and any potential for effects on non-target organisms.

2. Environmental Exposure

As with traditional chemical plant protection products the use pattern will determine the potential route and degree of exposure to the relevant environmental compartments. Certain use pattern could result in limited exposure and makes further exposure evaluation unnecessary. Disposal of waste water and spent soil needs consideration by stewardship, this is especially relevant for those microbial products that have the potential to produce spores which have the potential to remain viable for extended periods of time.

2.1. Fate and behaviour in the soil

For those products that are used exclusively in contained environments exposure to natural soils should normally not occur and hence further consideration of the fate and behaviour is not necessary. However, greenhouse soil is regularly exchanged (every few years) and often disposed of by spreading to normal fields. Similarly, waste water from closed systems may enter natural water bodies. Therefore, a decision for not evaluating fate and behaviour should be supported by a minimum amount of effect data.

Unlike traditional plant protection products microbes can proliferate in the environment. For obligate parasites the life cycle can only be completed in the presence of a suitable host and hence the vegetative life stages would not persist being subject to normal biotic and abiotic processes. Although spores may persist for a period of time they would not actively grow without the host and hence although we might expect a transient spike in CFUs this is of no real concern as numbers will rapidly decline. Also, for those organisms associated with plant roots the viability and hence persistence of those organisms without a suitable host will be limited. However, it should be noted that for certain genera of bacteria, notably bacillus has resistant spores that may persist for hundreds of years, consideration should be given to the potential for vegetative growth in the absence of a potential host. Generally sufficient information should be available in the open literature to estimate the potential for persistence in soil.

Furthermore, Methods for standard soil degradation studies are not appropriate for microbial products; if studies are deemed necessary, the study methodology must be adapted to the specific properties of these products, e.g. regarding application method, analytical method, or kinetic evaluation as the ‘degradation’ or decline of populations of micro-organisms does not follow first order kinetics.

A naturally occurring and indigenous organism will pose no additional risk to the environment if introduced into a system comparable to its natural environment at or below environmental background concentrations. The natural background concentrations of entomopathogenic fungi often heavily fluctuate depending on host densities and micro-climatic conditions. Similarly, concentrations found during naturally occurring epizootics (locally specific disease events in non-human environments) are often as high as those found after artificial inoculations (Kessler

2004[1]; Laengle 2005[2]). Therefore, relatively high and persistent concentrations of an indigenous organism in the environment, even if a result of artificial inoculation, does not necessarily add environmental risk.

For non-indigenous micro-organisms the concentration in the environment may also fluctuate through the season and persistence can also equate to prolonged exposure of potential non-target organisms. The ability of a non-indigenous species to establish itself permanently needs to be considered. Although an organism may be viable during the growing season it may be unable to overwinter and hence will only be a transient presence in the environment. If insufficient data is available from the literature on the behaviour of a non-indigenous species in a non-native environment then this would need to be further explored to limit the chance of introducing an "invasive species" with bespoke studies looking at the response of the microbial product to different environmental conditions.

If sufficient (exposure and/or effect) data are available from the literature to address any potential safety concerns then, from an industry perspective, no studies looking at the fate and behaviour in the environment need be conducted as part of a base package. The exception to this is if mammalian toxins (secondary metabolites) are produced, if studies on toxicity, pathogenicity, infectivity, or on ecotoxicology showed adverse effects or if the microbial product contains a non-indigenous species.

This document also covers extracts of microbial origin. These extracts may contain organic active substances that have the potential to impact on non-target species and which have their own characteristic physical and chemical properties and hence have a predictable behaviour in the relevant environmental compartments. To understand the potential behaviour of these compounds it is necessary to determine the soil persistence (DT50) and the KOC. A RASP study (Rapid Assay for Soil Persistence) has been the standard methodology employed to determine persistence but this does not provide an accurate estimation for biphasic compounds and hence other studies such as a Tub test should be considered. The standard procedures for assessment of chemical crop protection agents can support the selection of an appropriate study design. Based on the DT50 and KOC values, it is possible to reliably estimate the short-term and long-term behaviour of these active substances by model calculations, without need for further experimental data.

In summary the need to perform fate and behavioural studies in soil should be considered on a case-by-case basis, for living or attenuated organisms or spores, sufficient data should be available from literature and as a standard no studies are proposed. However, for secondary

[1] Kessler, P. (2004), 'Influence of Soil Factors on Virulence, Growth and Survival of the Fungus *Beauveria brongniartii*, a Specific Biocontrol Agent of the European Cockchafer (*Melolontha melolontha*)', Dissertation Thesis, Swiss Federal Institute of Technology Zurich, Department of Plant Pathology.

[2] Laengle, T. (2005), '*Beauveria brongniartii* (Sacc.) Petch as a Fungal Biocontrol Agent: Environmental Risk Assessment', Dissertation Thesis, Leopold-Franzens University Innsbruck, Department of Biology.

metabolites or microbial extracts with the potential for environmental exposure the following studies should be considered:

- Determination of the DT50 of the microbial product in soil.
- Determination of the KOC.

For microbial products that contain more than one (secondary) metabolite these studies should be considered for each compound.

2.2. Fate and behaviour in the water

Surface water

Microbial products may enter a receiving waterbody via drift, surface run off, drainage or via an infected host. Generally, microbial products are chosen to control pests and diseases either on the phyllosphere or the rhizosphere and are therefore unlikely to propagate in the aquatic environment, indeed they may be utilized as food by aquatic organisms. Furthermore, native microorganisms will regularly enter receiving bodies and their use as a plant protection product is unlikely to significantly impact surface water concentrations. A safe use should normally be able to be determined without generating data by reference to literature and consideration of the nature, selectivity and toxicity of the microbial in question.

Ground water

As with surface water, native microorganisms may enter ground water and their use as plant protection products should not significantly impact ground water. A safe use should normally be able to be determined without generating data by reference to literature and consideration of the nature, selectivity and toxicity of the microbial in question.

As above the exception to this is if mammalian toxins (secondary metabolites) are produced, if studies on toxicity, pathogenicity, infectivity, or in ecotoxicology showed adverse effects or in case of a non-indigenous species, or where the microbial product is a microbial extract. For secondary metabolites or microbial extracts sufficient information needs to be available to ascertain the potential concentrations in ground water and hence soil degradation rates (DT50) and KOC determination should be conducted if the information is not readily available in the literature. These substance properties can be used to determine the potential concentrations in all relevant compartments (soil, ground water etc.).

3. Ecotoxicology

The relevant endpoints can be addressed either by literature information, history of safe use, screening and/or guideline studies. OPPTS numbers are given to indicate what endpoint (knowledge need) is required. During a development project this can be a step-wise process.

3.1. Birds and mammals

The protection goals for higher tier vertebrates are the protection of the individual and hence this may lead to additional data requirements. A bird pathogenicity study may be needed unless pathogenicity can be excluded based on literature evidence or exposure is negligible based on use pattern, specific biological properties or host specificity.

Potential hazards resulting from exposure to birds can be identified by the avian oral toxicity study (OPPTS 885.4050) on the TGAI. Additionally, if inhalation is a relevant exposure route an avian inhalation toxicity test may be conducted with the TGAI (OPPTS 885.4100).

With respect to safety to mammals, reference should be made to the human health assessment with potential hazards being identified by the rat acute oral toxicity/pathogenicity study (OPPTS 885.3050) with the TGAI. If concerns are raised about the toxicity of any component in the end-product then an acute oral toxicity study (OPPTS 870.1100) with the end-product may be conducted. These are the appropriate data with which to evaluate pathogenicity/toxicity to wild mammals.

In summary, the following studies would enable a safety assessment for microbial products.

- Where exposure and/or pathogenicity cannot be excluded based on available data, an avian oral toxicity study (OPPTS 885.4050) on the TGAI should be conducted

- If the inhalation route is relevant, an avian inhalation toxicity test may be conducted with the TGAI (OPPTS 885.4100)

3.2. Aquatic organisms

If the proposed use of the product precludes entry into a receiving waterbody then it may be justified to not conduct any aquatic toxicity studies. However, when safety for other uses needs to be assessed a minimum dataset should be considered. To demonstrate safety, fish toxicity (OPPTS 885.4200) may be performed with the TGAI, however consideration should be given to literature and the selectivity of the microbial and given ethical concerns a safe use may be concluded without the generation of experimental data. A test should be conducted with an aquatic invertebrate (*Daphnia magna*), again with the TGAI (EPA may update the guideline to take into account possible secondary effects unrelated to toxicity). Although the base dataset should suffice for the vast majority of microbial products, it may be prudent to consider additional algae and macrophyte testing on a case by case basis (e.g. herbicidal or phytopathogenic).

Due to low exposure and bridging from freshwater testing, testing with estuarine and marine fish and invertebrates is not generally necessary. The following studies would enable a safety

assessment for microbial products. These studies are only relevant when exposure to the aquatic environment is predicted or when natural exposure to an indigenous microbial product at comparable levels cannot be demonstrated.

- Study with *Daphnia magna* (OPPTS 885.4240)
- Green algal toxicity test (OECD 201) - this should only be considered for herbicides or microbial products related to plant pathogens.

A fish toxicity/pathogenicity study would not normally be conducted, unless indicated by literature data or significant exposure.

3.3. Bees and non-target arthropods

A number of biological pesticides are developed to control insect pests. Hence, for these products consideration needs to be given to the selectivity and the proposed use pattern. As a minimum a honeybee study should be conducted (OPPTS 885.4380 / OECD 213/214) with either the TGAI or the end-product. Results of this study, data from the efficacy package or from literature may raise concerns around safety of non-target arthropods other than bees. Such situations may need to be addressed on a case by case basis. Especially for entomopathogenic fungi data on more than one test species should be considered to conclude on the safety of the microbial product. If the use pattern precludes exposure to bees (indoor, soil, BBCH stage, non-attractive crop) then it may be possible to determine safety without reference to bee toxicity data. The following studies should be conducted to enable an assessment of the safety of the microbial product.

- Bee test (OPPTS 885.4380 / OECD 213/214)
- For insecticidal foliar microbial products information on the effects on *Typhlodromus sp* and/or *Aphidius sp* (or equivalent species) should be considered.

3.4. Soil organisms

The soil is a dynamic environment with a range of micro and macro-organisms interacting to contribute and deliver soil processes. For indigenous species soil exposure will occur naturally and any increased exposure through application of the microbial product is unlikely to have significant impact on the soil function. Furthermore, uses that preclude exposure will have no impact and hence safety may be assessed without reference to study data.

For non-indigenous species or where there is no evidence of natural exposure of the microbial product to the soil further understanding on the potential effects on soil organisms needs to be

considered. As part of this consideration the selectivity and mode of action of the microbial product needs to be considered. For highly selective microbial products, even those that are targeting insects or nematodes safety can be concluded without conducting studies. For those microbial products for which a safe use cannot be determined from already available information then effects on non-target soil organisms need to be considered.

The standard test organism is the earthworm. The standard test is an acute toxicity study (OECD 207); however, if acute studies indicate effects on biomass or mortality a reproduction study (OECD 222) may be needed.

For insecticidal microbial products consideration should also be given to potential effects on collembolans (OECD 232). Similarly, for miticides investigation of effects on the soil mite *Hypoaspis* (OECD 226) should be considered. The Soil Microbial organism (SMO) study (OECD 216) may be considered as well for any substance, if applicable. These studies are not part of the base safety data package, but should be considered if evidence from literature, efficacy studies or other ecotoxicology studies indicates that there may be an issue. Where the microbial product is developed to control soil organisms then additional consideration on the impact of the use to the soil microbial community needs to be considered.

3.5. Non-target plants

Given that microbial products are intended to be used as plant protection products it should be possible to provide a reasoned case using proprietary efficacy data and open literature to assess safety to non-target plants (NTPs). Where microbial products have been introduced to control weeds, e.g. allelopathic bacteria or where the microbial product is related to a plant pathogen more consideration will be needed. Reference to literature and efficacy data should provide sufficient information to determine safety. Effects in crop can be predicted from efficacy trials and hence additional studies are not normally required. If adverse effects are observed in the efficacy trials a Tier 1 NTP screen should be considered.

4. Secondary metabolites

Secondary metabolites can be defined as organic compounds produced by an organism that are not directly involved in the primary function of the organism.

Some microbes, e.g. of the genera, *Clostridium*, *Bacillus*, *Trichoderma* and others are known to produce secondary metabolites that are of toxicological concern. This raises concerns about the potential for intended microbes in products to produce secondary metabolites either in the product or in the environment following application. A literature review should be undertaken to establish if the product organism, or a closely related organism, produces secondary metabolites. In the absence of existing information about possible secondary metabolites, the

above study package would be assumed to cover the environmental properties and no further work needs to be conducted.

If there is sufficient reason to believe that secondary metabolites of concern could exist in the preparation or may be produced in the environment following application, additional testing may be necessary. A better understanding on the nature of the metabolite and its natural occurrence/background level will be beneficial and consideration of any additional testing will need to be done on a case-by-case basis to determine the impact on the environment. Given that secondary metabolites can be considered more akin to a classical chemical plant protection product a better understanding of the exposure profile (may need fate studies; DT_{50} and adsorption) and the potential impact on non-target organisms will help to determine safety of the microorganism under field conditions. Acute toxicity studies with the TGAI or the formulated product are usually suitable to evaluate toxicity to non-target organisms.

A secondary metabolite can be an antibiotic. If it is known to be a clinically relevant antibiotic it needs special attention.

Major change in manufacturing process/conditions may affect the secondary metabolite profile which may require special attention. Additional safety studies may have to be considered.

5. Risk assessment

If a lack of hazard from the microbial product has been demonstrated in above described hazard tests no quantitative risk assessments are required.

Having said this, as part of the evaluation process it should be identified whether there are existing routes of environmental exposure to the microbe either via an existing MPCA or due to natural occurrence. This will provide a broader understanding of the environmental exposure and importantly whether any adverse incident reporting is likely.

If a lack of hazard has not been demonstrated in the Tier 1 hazard tests, quantitative exposure estimations and risk assessments will be required (in addition to higher Tier hazard evaluations).