

GUIDELINES

Framework for Assessing the Human Safety of Microbial Pesticides CropLife International's Guidelines, established January 2018

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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 Archaeobacteria; 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 human 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.

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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 human safety for microbial pesticides

The following studies are generally required for the registration of microbial pesticides; however, there are some differences between the regulations in various countries/regions.

Description	Test Material	Guideline Reference
Acute oral toxicity/pathogenicity	TGAI	OPPTS 885.3050
Acute pulmonary toxicity/pathogenicity	TGAI	OPPTS 885.3150
Acute injection toxicity/pathogenicity	TGAI	OPPTS 885.3200
Cell culture (for virus)	TGAI	OPPTS 885.3500
Acute oral toxicity	EP	OPPTS 870.1100
Acute dermal toxicity	EP	OPPTS 870.1200
Acute inhalation toxicity	EP	OPPTS 870.1300
Acute eye irritation	EP	OPPTS 870.2400
Acute dermal irritation	EP	OPPTS 870.2500
Skin sensitisation	EP	OPPTS 870.2600

TGAI – Technical Grade Active Ingredient

EP – End-use Product

It should be noted that in cases where the End-use Product (EP) is similar to the Technical Grade AI (TGAI) and/or contains only toxicologically inert co-formulants the acute oral, dermal and inhalation studies on the EP are often not required.

Genotoxicity tests on microbial products are generally not required by regulatory agencies.

Where treatment related effects are seen in these Tier 1 studies, additional testing could be required.

It should be noted that this framework for assessing the human safety of microbial pesticides may at times require more or less data than required by country specific regulatory requirements.

Safety standards and evaluation

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 can provide this information. An appropriate set of screening studies and/or guideline studies as outlined below will provide the necessary knowledge to properly 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). Additionally, 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 need to be appropriate to the species being produced e.g. specific or analogous strains. Basic information regarding the identity and characteristics of the microbial product can be useful in predicting toxicological or pathological properties and behaviour. A microbial product that belongs to a group of microorganisms that have never been found in association with mammalian disease may require the minimum required studies to confirm safety, whereas a microbial product that is taxonomically similar to a clinically significant microorganism could require further examination, perhaps by conducting additional studies. This basic information, along with any documented history of safe use and other literature information, adds to a weight of evidence that can be used to evaluate product safety.

2. Hazard evaluation of microbial products

Users of microbial products will likely have some level of external exposure via the dermal route as well as internally via the respiratory route. If the product is being used on food producing crops, consumers could have some level of exposure through the diet. Each exposure scenario is discussed in further detail below and a framework of study requirements to assess the hazard

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is proposed. In addition, systemic exposure could occur where epithelial barriers are compromised.

As a first step, a literature search should always be conducted to understand the data that is available in the public domain.

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

Oral exposure

Potential hazards resulting from oral exposures are identified by the acute oral toxicity/pathogenicity study (OPPTS 885.3050) of the TGAI and the acute oral toxicity study (OPPTS 870.1100) of the EP.

As a minimum, the acute oral toxicity/pathogenicity study of the TGAI is required to assess safety. The acute oral toxicity study of the EP is not required but may be needed if the toxicity of co-formulants are unknown or of concern. If studies on both the TGAI and the EP are available both sets of results should be taken into account.

Dermal exposure

Potential hazards resulting from dermal exposures can be identified by either the acute dermal toxicity/pathology study (OPPTS 885.3100) on the TGAI or the acute dermal toxicity study (OPPTS 870.1200) on the EP. There is very limited difference between these two studies.

As a minimum the acute dermal toxicity study on the EP is required to assess safety.

Inhalation exposure

Potential hazards resulting from inhalation exposures are identified by the acute pulmonary toxicity/pathogenicity study (OPPTS 885.3150) of the TGAI and the acute inhalation toxicity study (OPPTS 870.1300) of the EP.

To assess safety, as a minimum the acute pulmonary toxicity/pathogenicity study on the TGAI may be required if indicated by relevant information existing in the public domain or the properties of the AI and/or use pattern indicate a significant exposure risk. The acute inhalation toxicity study of the EP is not required but may be needed if the toxicity of co-formulants are unknown or of concern. If studies on both the TGAI and the EP are available both sets of results should be taken into account.

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Systemic exposure

The acute injection toxicity/pathogenicity study (OPPTS 885.3200) on the TGAI is a key study as this assesses infectivity and pathogenicity if the epithelial barriers are bypassed. This study may be required to assess safety for systemic exposure if indicated by relevant information existing in the public domain.

Genotoxicity

In general, genotoxicity studies are not required for the safety assessment of microbial pesticide products. However, microorganisms may produce secondary metabolites with genotoxic potential. Therefore, on a case by case basis, appropriate genotoxicity studies may have to be conducted to confirm safety.

Repeated dose toxicity

Repeat dose toxicity studies are usually not needed. However, this needs to be assessed on a case by case basis on above acute toxicity, pathogenicity and infectivity information, knowledge on secondary metabolites and other (literature) information.

3. Position on eye and dermal acute irritation studies

The acute eye and dermal irritation studies (OPPTS 870.2400 and OPPTS 870.2500, respectively) are likely to be required for hazard classification purposes and for safety purposes to assess the need for PPE for users of the EP.

4. How much of a microbial product are people exposed to?

If a lack of hazard from the microbial product has been demonstrated in guideline studies at limit doses, then it is not necessary to estimate exposure levels either to end-use operators or to consumers via the diet. Under these circumstances no quantitative human risk assessments are required.

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

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

5. How should the potential for sensitization be managed?

Certain microbial products may have the potential to be human sensitizers, either via the dermal and/or the inhalation routes and this is therefore an important area to manage. It is possible to experimentally test for skin sensitization, although currently there are no validated test methods or guidelines to assess sensitization of microbial products. Therefore, the relevance of these tests for microorganisms is debatable.

6. Secondary metabolites

Secondary metabolites can be defined as organic compounds produced by an organism that are not directly involved in the normal growth, development or reproduction of that 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 the issue 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 toxicity study package would be assumed to cover the toxicological properties and no further work needs to be conducted if no adverse effects are observed at limit dose levels.

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, then the acute exposures during Tier 1 toxicity studies may be insufficient to identify hazards. Additional testing requirements would need to be assessed on a case-by-case basis once the secondary metabolites have been identified and characterized. Further hazard testing could include genotoxicity or higher tier, repeat exposure studies.

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

Additionally, a significant change in manufacturing process and/or conditions may affect the secondary metabolite profile which may require special attention, and further safety studies may be required.

7. Antibiotic Resistance

Antibiotic resistance is widely spread in natural microorganism populations including organisms used in food and feed and in agriculture. It is not considered a fundamental safety concern requiring testing.

Conclusions

The following guideline studies should be considered based on use pattern, test material, knowledge on biological properties. At least one of the following may be needed.

- Acute oral toxicity/pathogenicity OPPTS 885.3050
- Acute pulmonary toxicity/pathogenicity OPPTS 885.3150
- Acute injection toxicity/pathogenicity OPPTS 885.3200

In addition, the following study on the EP is also required

- Acute dermal toxicity OPPTS 870.1200

Additional studies on the EP may be needed if co-formulants are considered of concern.

It may be possible to use studies conducted with similar strains of microbes if it can be justified that the strains are equivalent to those in the TGAI. The justification of equivalency would require careful case-by-case consideration.

When a lack of toxicity, infectivity and pathogenicity is demonstrated, a weight of evidence evaluation can conclude that there is no mammalian hazard arising from the use of that microbial product.

If adverse effects are observed in the studies mentioned above, additional hazard data will likely be required (e.g. higher tier studies using repeat exposures). From a safety perspective this may be manageable, however it is unlikely that a microbial product with adverse effects would be progressed due to cost and brand/image reasons. Further consideration of these situations will be given when such an example arises.