CropLife International Biologicals Project Team

This document reflects a consensus of the views of members of the Biological Products Team (BPT) of CropLife International. The BPT recognises that technologies and regulations are in continual flux and the document will evolve to reflect these changes

Principles for the approach to safety assessments of living Microbial Products

Microbial products are often considered to have a low risk profile. The registration process for these products are typically based on this assumption and use a stepwise (tiered) evaluation to identify hazards which may affect the risk assessment. Typically, registered microbial technical grade active ingredients (TGAIs) have favourable risk profiles. Hence, the required registration data are usually considerably less and more targeted than for chemical pesticides, and in some cases qualify for 'fast-track registration'.

In this document microbial pesticides are defined as microbial agents 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; or
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.

The safety assessment must include the following for microbial product registrations:

Tiered data development and risk assessment. If Tier 1 information raises concerns, then the Tier 2 assessment may be triggered.

1. **Identification, taxonomic and genetic characterisation at strain level.**
    - Genetics, morphology and biochemistry may all be used to ensure characterization
    - The strain needs to be deposited into an international strain bank.

2. **Mode of Action**

    A literature review and, if necessary, studies to identify possible metabolites formed by organism.

    - Targeted, hypothesis driven genetic characterisation may be useful to provide evidence that the microbe does not produce metabolites of concern.
    - Known secondary metabolites may have biological profiles already described. They may fall outside of the applicability domain for QSAR assessment due to structural complexity.

3. **Screening/guideline studies provide knowledge to assess the safety of the organism and the plant protection product.**

    - These should be appropriate to the species being produced, *e.g.* specific or analogous strains.
    - Available scientific literature can be useful to predict toxicity and pathogenicity.
    - Screening guideline studies usually contain the microbial metabolites, depending on the concentration these may give a clue as to possible metabolites of concern which may require further testing.

4. **Quality control measures ensure no contamination by potential pathogens**

    - Determine the presence/absence of unintended microbial contaminants [see OECD Microbial Contaminant Limits for Microbial Pest Control Products (OECD 2011)].
    - Additional quality control measures may be required if production conditions permit growth of human or animal pathogens, e.g. *Enterobacteriaceae*.

5. **Documented history of safe use and other literature information**

    - Data are commonly available from the open literature, but relevance and quality must be evaluated and justified.
    - Evidence of widespread exposure may support safe use, particularly that existing natural exposure is comparable to exposure from the uses being supported. Note that topical foliar treatment is not analogous to natural exposure to a soil microbe
    - Strain-specific studies can be considered if relevant to the use pattern.

CropLife International proposal for TIER 1

In the case where the Technical Grade Active Ingredient (TGAI) cannot be tested for a technical reason, tests on the End Use Product (EUP) would be acceptable (and *vice versa*). For example, some microorganisms are manufactured in a closed system from inoculation with the starter material to the formulated product so it is not possible to have samples of the TGAI.

	Requirement	TGAI	EUP	Notes
1	**Biological/Chemical Properties**			
1.1	Genus, species, strain/isolate	R		
1.2	Form (whole organism, endospore, extract, etc.)	R		
1.3	Biological properties including Source/origin, host range, native/non-native	R		If non-native, consider the potential to become native (see 4.1).
1.4	Physical properties, including pH (CIPAC MT75.3), viscosity OECD 114, CIPAC MT192), influence of the environmental parameters on growth [temperature/humidity].		R	
1.5	Mode of action: e.g. infection of target, competitive or antagonistic behaviour, etc., relevance of secondary metabolites.	R		
1.6	Genetic stability and potential for gene transfer.	R		
1.7	Assessment for anti-microbial resistance (AMR) genes and other potential genes of concern.	R		If AMR genes are on a mobile genetic element, the risk of Horizontal Gene Transfer should be demonstrated (see 1.6)[1].
1.8	Manufacturing process.	R	R	The manufacturing process for both the TGAI and EUP should be described. If the fermentation process is integrated and no TGAI can be isolated, then a description of the integrated process to produce the TGAI/EUP is required. Note that different products containing the same strain may have different manufacturing processes.

TGAI technical grade active ingredient, EUP end use product. (see note on terminology at the end of the paper), R required, CR conditionally required.

[1] For fungi and viruses there is no need to assess the potential transfer of genes for resistance to antimicrobials. Horizontal transfer of AMR genes between fungi appears to be very rare and is not associated with specific mechanisms.

	Requirement	TGAI	EUP	Notes
1.9	Analytical methods: identification of strain, assay.	R	R	Strict adherence to specific analytical methods is required. Transfer of analytical method (e.g., colony forming unit assay) to outside laboratories can be problematic. Slight changes in conditions can lead to different results – for example, variation in colony forming unit (CFU) assay results are normal.
1.10	Specification of technical product: minimum assay, chemical impurities, microbial contaminants.	R		Parameters should be presented as ranges (i.e. upper and lower limits).
1.11	Specification of formulated product, minimum assay, chemical impurities, microbial contaminants, co-formulants, formulation type.		R	Parameters should be presented as a range except minimum assay. If biocides are included to facilitate storage stability, they should be checked for conformity with organic agriculture.
1.12	Storage stability (CIPAC MT46.3, MT39.3).		R	
2	**Toxicity/ Pathogenicity to Mammals**			
2.1	Infectivity, pathogenicity and host specificity (living microorganism).	R	CR	Data from the literature. Laboratory studies are necessary if there is insufficient evidence (see end points below). If it is impossible to use the TGAI for testing (e.g. spore powder) and the EUP does not contain co-formulants which affect its toxicity, then testing may be accepted with the formulation.
2.2	Toxicity (Secondary metabolites, including clinically relevant antimicrobials; impurities, contaminants, co-formulants).	R	R	Data from the literature. Laboratory studies are necessary if there is insufficient evidence (see end points below).
2.3	Acute oral toxicity/pathogenicity (OPPTS 885.3050).	R		
2.4	Acute pulmonary toxicity/ pathogenicity (OPPTS 885.3150).	R		
2.5	Cell culture (for virus only) (OPPTS 885.3500).	R		

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	Requirement	TGAI	EUP	Notes
2.6	Acute oral toxicity: • No specific microbial guideline. Testing based on OPPTS 870.1100 or OECD 425.		CR	
2.7	Acute dermal toxicity: • No specific microbial guideline. Testing based on OPPTS 870.1200 or OECD 402.		CR	
2.8	Acute inhalation toxicity: • No specific microbial guideline. Testing based on OPPTS 870.1300 or OECD 403.		CR	
2.9	Acute eye irritation • No specific microbial guideline. Testing based on OPPTS 870.2400 or OECD 405.		CR	
2.10	Acute dermal irritation: • No specific microbial guideline. Testing based on OPPTS 870.2500 or OECD 404.[2]		CR	
2.11	Skin sensitisation		*	Discussion point at OECD. May be required depending on co-formulants. in case needed OPPT 870.2600 or OECD.

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[2] The calculation method may be used in some regions.

	Requirement	TGAI	EUP	Notes
3	**Toxicity/pathogenicity to Non-Target Organisms**			
3.1	Non-target effects (Secondary metabolites, including clinically relevant antimicrobials; impurities, contaminants, co-formulants) • Data from the literature • Relevance for non-target organisms • Studies if insufficient evidence (below)	R	R	Route of exposure / use should be considered.
3.2	Avian oral toxicity/pathogenicity (e.g. OPPTS 885.4050).	R		Data from mammalian testing should be considered prior to undertaking this testing. Consideration should also be given to the end use of the product.
3.3	Avian inhalation toxicity/ pathogenicity (e.g. OPPTS 885.4100).	CR		Typically waived; conducted only under very specific circumstances. TGAI may be modified to facilitate administration as some biologicals cannot be administered as TGAI.
3.4	Freshwater fish toxicity/ pathogenicity (e.g. OPPTS 885.4200).	CR		Histopathological and blood portion of assay need not be conducted if pathogenicity is not suspected. If the TGAI is an obligate microbe, consider a waiver for this based on vertebrate testing.
3.5	Freshwater Cladoceran (*Daphnia magna*) toxicity/pathogenicity (e.g. OPPTS 885.4240).	CR		The US EPA guideline study is to be updated for microbial products.
3.6	Estuarine/marine animal toxicity/pathogenicity (e.g. OPPTS 885.4280).	CR		Typically waived; conducted only under very specific circumstances
3.7	Algal toxicity. No specific microbial guideline. Testing based on OCSPP 850.4500 or OECD 201.	CR		Typically waived; conducted only under very specific circumstances
3.8	Aquatic macrophyte toxicity No specific microbial guideline. Testing based on OCSPP 850.4400 or OECD 221.	CR		Typically waived; conducted only under very specific circumstances

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	Requirement	TGAI	EUP	Notes
3.9	Honey bee toxicity (oral/contact) No specific microbial guideline. Testing based on OECD 213/214.	R	R	Either TGAI **or** EUP
3.10	Honey bee pathogenicity OPPTS 885.4380.	R	R	Either TGAI **or** EUP
3.11	Non-target arthropods toxicity/pathogenicity (other than bees) OPPTS 885.4340.	R	R	Either TGAI **or** EUP. May not be required in some regions.
3.12	Earthworm toxicity No specific microbial guideline. Testing based on OCSPP 850.3100 or OECD 207 or 222.	R	R	Either TGAI **or** EUP
4	**Environmental impact**			
4.1	Natural background levels in the environment as reported in the literature	R		Usually, the microorganisms introduced as biocontrol agents already exist in the environment, and application leads to transient changes in the composition of the (soil) microflora. The specific requirements vary between countries.
4.2	If non-native species, can it become native? • Literature review and risk assessment.	R		As most bacteria and fungi are ubiquitous, there may be literature to demonstrate its presence. If not, then related species or species with comparable characteristics can be used to assess the risk of becoming native and affecting non-target native species.
4.3	Mobility in the environment • Literature review if necessary	R		For microbes there is usually no need for investigation of degradation and movement within and between compartments if the risk of spread is tested with host range, infectivity, etc. Further investigation may be needed if there are secondary metabolites. If necessary, a literature review should encompass the natural background levels, the known native geographic range and mobility in the environment.

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	Requirement	TGAI	EUP	Notes
4.4	Fate of secondary metabolites in the soil			If literature is insufficient, consider undertaking a Ready Biodegradability OECD 301 and Activated sludge OECD 303 tests that might show the secondary metabolite is not persistent. If the metabolite is persistent then conduct a predictive toxicity assessment.
5	**Efficacy**			
5.1	Indicate target pest(s)		R	In some cases, it may be possible to extrapolate data between crops for the same target pest
5.2	Experience with the product elsewhere		R	
5.3	Confirmation of claims of target specific action and potency: • screening (laboratory) studies if insufficient data		R	
5.4	Evidence of effects in the field if insufficient data • field trials (EPPO guidelines or similar)		R	
6	**Packaging and Labelling**			
6.1	Packaging materials, pack sizes.		R	
6.2	Storage information.		R	
6.3	Safety data sheet.		R	
6.4	Label meeting requirements of the competent authority.		R	Include effectiveness and use with IPM.

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TIER 2

2.12	**Human Health Exposure/ Environmental Fate and Effects Data** If any results from tier 1 suggest further risk assessment.			• Extrapolation to human health can be done from mammalian testing if the microbial pest control agent is in any category of concern. **The active agent will be rejected if it is a true human pathogen** (i.e. excluding results from immuno-compromised individuals). • Results from routine general monitoring programs and health surveys of 'worker's safety' at the production site and from the literature must be used to assess general human health risks, noting that it can be difficult to be as specific for microbials as is expected for chemical pesticides.
	Add examples of studies/information.			
7	**Residue Data** • Only relevant if toxic residues of the active agent of any kind are likely and to be expected on food or feed items. • Substances used for formulation must not produce residues on feed or food items. This must be documented by relevant references.			• A waiver statement is needed. TGAIs which have proven to be non-toxic, non-infecting, and non-pathogenic are not considered to require a residue limit (*i.e.* exempt from tolerance) unless specifically requested by the regulator. • Microbial secondary metabolites are usually readily biodegradable. If there are toxic residues, then studies may be required unless the residues can be demonstrated to be not persistent.
8	**Additional Data Requirements**			
	As necessary according to the outcome of the risk assessment.			

Additional resources from CropLife International

Microbial-Framework-for-Environmental-Safety-FINAL.pdf (croplife.org)

Microbial-Framework-for-Human-Safety-FINAL.pdf (croplife.org)

Position Paper on Regulatory use/submission of Whole Genome Sequencing and Bioinformatics *(currently available only to BPT members)*

Biological Crop Protection Industry Message Map *Draft update 27 July 2021 (currently available only to BPT members)*

Note on terminology:

Different terms are used by different agencies and authors. Technical grade active ingredient (TGAI) is equivalent to the technical grade of a microbial pesticide agent (MCPA). End use product (EUP) is equivalent to microbial pest control product (MPCP).

Agency with link	**The micro-organism (e.g. bacterium, fungus, protozoan, virus, viroid, mycoplasma, algae)**	**The micro-organism (e.g. bacterium, fungus, protozoan, virus, viroid, mycoplasma, algae) and any associated metabolites/toxins, fermentation residues and contaminants as manufactured**	**A product formulated and ready for sale**
This position paper		Technical Grade active ingredient (TGAI)	End Use Product (EUP)
FAO	**Microbial pest control agent (MCPA)** A microorganism (protozoan, fungus, bacterium, virus, or other microscopic self-replicating biotic entity) (revised ISPM Pub. No. 3. IPPC, 2005) and any associated metabolites, to which the effects of pest control are attributed (OECD, 2008). A microorganism active substance may contain viable and/or non-viable microorganisms. It can contain relevant metabolites/toxins produced during cell proliferation (growth), material from the growth medium, provided none of these components have been intentionally altered. *[Equivalent to Pure Technical (TC) for chemical pesticides]*	**Technical Grade of microbial pest control agent (MCPA)** Microbial material used for manufacture of microbial pest control products. It is the purest preparation of the MPCA resulting from a typical production process and contains no additives except for purposes of MPCA growth or replication, or typical purification and preparation. It may be commercially distributed to manufacturers of microbial pest control products either in its pure form or augmented with preservatives, stabilizers, and diluents; or it may be a hypothetical stage in the manufacture of the microbial pest control product. *[Equivalent to Technical*	**Microbial pest control product (MPCP)** A product containing an MPCA that is registered or labelled with instructions for direct use or application for pest control purposes.

		Concentrate (TK) for chemical pesticides]	
FAO	MCPA can be defined as: A microorganism (protozoan, fungus, bacterium, virus, or other microscopic self-replicating biotic entity) (revised ISPM Pub. No. 3. IPPC, 2005) and any associated metabolites, to which the effects of pest control are attributed (OECD, 2008). A microorganism active substance may contain viable and/or non-viable microorganisms. It can contain relevant metabolites/toxins produced during cell proliferation (growth), material from the growth medium, provided none of these components have been intentionally altered		MPCP can be defined as: A product containing an MPCA that is registered or labelled with instructions for direct use or application for pest control purposes.
OECD		TGAI technical grade active ingredient.	
OECD OECD	**MPCA Microbial pest control agent – Microorganism active substance**		**MPCP Microbial pest control product**