

# Guidance on the Biocidal Products Regulation

Volume V, Guidance on Active Micro-organisms and Biocidal Products

Version 2.1  
March 2017



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### **Guidance on the Biocidal Products Regulation: Volume V - Guidance on active micro-organisms and biocidal products**

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## Document History

Version	Comment	Date
Version 1.0	First edition	August 2015
Version 2.0	<p>Full revision of the guidance to address in particular a series of open issues identified during the first consultation and the conclusions reached at the Ctgb "Workshop on harmonisation of the toxicological risk assessment for micro-organisms used in plant protection" held (12-13 November 2015).</p> <p>The update includes the following:</p> <ul style="list-style-type: none"> <li>- Merging of sections 1 and 3 in a new introductory section 1. Inclusion of the considerations on secondary metabolites agreed during the workshop and indication that the guidance does not address primary and secondary metabolites separately. Stressed the recommendation to validate the methods.</li> <li>- Section 2: further clarification of the applicability for micro-organisms (MOs) of legal definitions of "Active substance" and "Substance of concern". Clarification of the definition of "pathogenicity".</li> <li>- Section 3: deletion of the reference to indigenous/not indigenous status of the MOs; Inclusion of reference to biopotency as potentially useful to characterize the biocidal effect; clarification about the need to indicate the maximum content of MO in the TGAI in case is needed for risk assessment purposes; clarification about the need for methods validation; revision of endpoint 3.2 to clarify the actual limited relevance of endpoints colour, odour and effects of light; update of the references to the CIPAC Methods; clarification about the sensitisation potential to be assumed in absence of scientific evidence; clarification about sensitive target groups, for which the risk needs to be assessed case-by-case.</li> <li>- Section 4: indication that need for non-target organisms test should be considered case-by-case; update of endpoint 7.7 "number and timing of applications and duration of protection".</li> <li>- Section 5: clarification about vulnerable group to be considered case-by-case; clarification about spores, to be considered according to the case; clarification about sensitising potential; clarification about long clearance time which is not automatically associated to harmful effects; stressed the need to avoid animal testing as far</li> </ul>	August 2016

Version	Comment	Date
	<p>as possible; removed the indication that models for chemicals can be used with MOs due to the very limited applicability and stress on qualitative or semi-quantitative approaches; inclusion of missing text for section 5.2.2.2.5; clarification about not existence of harmonisation regarding the prescription of PPE.</p> <ul style="list-style-type: none"> <li>- Section 6: inclusion of clarification about the use of relevant units; clarification that the assessment is more likely to be qualitative or semi-quantitative than quantitative.</li> </ul> <p>Clarification throughout the document that metabolites/toxins should be considered only when relevant.</p>	
Version 2.1	<p>Corrigendum to add text and links on "Applicability of Guidance"</p> <p>The text has been revised as follows:</p> <ul style="list-style-type: none"> <li>• Preface: to add text and links on "Applicability of Guidance"</li> </ul>	March 2017

## Preface

This Guidance is to be applied to applications for active substance approval and product authorisation as submitted under the Biocidal Product Regulation (EU) No 528/2012 (the BPR). This document describes the BPR obligations and how to fulfil them.

This Guidance replaces the addendum “Technical Notes for Guidance on data requirements for micro-organisms including viruses and fungi (EU, 2005)”, to the Technical Notes for Guidance (TNSG) on Data Requirements (EU, 2008a) in support of Directive 98/8/EC (Biocidal Product Directive - BPD).

### Applicability of Guidance

Guidance on applicability of new guidance or guidance related documents for active substance approval is given in the published document “*Applicability time of new guidance and guidance-related documents in active substance approval*” available on the BPC Webpage<sup>1</sup> [<https://echa.europa.eu/about-us/who-we-are/biocidal-products-committee>] and for applicability of guidance for product authorisation, please see the CA-document CA-july2012-doc6.2d (final), available on the ECHA Guidance page [[https://echa.europa.eu/documents/10162/23036409/ca-july12-doc\\_6\\_2d\\_final\\_en.pdf](https://echa.europa.eu/documents/10162/23036409/ca-july12-doc_6_2d_final_en.pdf)].

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<sup>1</sup> Link available under Working Procedures (right column) [<https://echa.europa.eu/about-us/who-we-are/biocidal-products-committee>]

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## List of Abbreviations

Standard term / Abbreviation	Explanation
ADI	Acceptable Daily Intake
ADS	Additional data set
BPR	Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products (Biocidal Products Regulation)
CDS	Core data set
CFU	Colony forming unit
CIPAC	Collaborative International Pesticides Analytical Council
CLP	Regulation (EC) No 1272/2008 on Classification Labelling and Packaging of substances and mixtures
CTGB	Board for the Authorisation of Plant Protection Products and Biocides
DNA	Deoxyribonucleic acid
ECHA	European Chemicals Agency
EED	Estimated environmental density
EFSA	European Food Safety Authority
ENED	Estimated no effect density
EPPO	European and Mediterranean Plant Protection Organization
FAO	Food and Agriculture Organization of the United Nations
HACCP	Hazard Analysis and Critical Control Points
ISO	International Organization for Standardization
IU	International Unit
ITU	International Toxic Unit
OECD	The Organisation for Economic Co-operation and Development
PEC	Predicted exposure concentration
PNEC	Predicted no effect concentration
PNED	Predicted no-effect density
PPE	Personal protection equipment
rRNA	Ribosomal ribonucleic acid
REACH	Regulation (EC) No 1907/2006 concerning the Registration Evaluation Authorisation and Restriction of Chemicals (REACH)
TGAI	Technical grade of active ingredient
TGD	Technical Guidance Document
TNsG	Technical Notes for Guidance

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US EPA	United States Environmental Protection Agency
UVCB	Substances of Unknown or Variable composition, Complex reaction products or Biological materials
VBNC	Viable but non-culturable bacteria
YOPI	Young, old, pregnant and immune-compromised persons

## 1. Introduction

### 1.1 Scope of the guidance

The aim of this guidance is to substantiate the requirements of Annex II Title 2 (Information Requirements for Active Substances, Micro-organisms) and Annex III Title 2 (Information Requirements for Biocidal Products, Micro-organisms), of the BPR for the preparation and evaluation of dossiers of active micro-organisms at strain level.

As the judgements made by the Competent Authorities for Biocides during the evaluation and decision-making process must be based on scientific principles, preferably recognised at international level, advice from relevant experts on micro-organisms may be necessary.

Micro-organisms have their own biology and response to the environment. It is therefore important to have knowledge about the biological properties of the actual micro-organism.

Due to the ability of micro-organisms to proliferate, there is a clear difference between chemicals and micro-organisms used as biocidal products. Hazards arising are not necessarily of the same nature as those presented by chemicals, especially in relation to the capacity of micro-organisms to persist and multiply in different environments. These differences between micro-organisms and chemicals should be taken into account in the assessment. Moreover, micro-organisms consist of a wide range of different organisms, often isolated from the environment, all with their own unique characteristics, behaviours in different environments and modes of action.

The active micro-organism in the biocidal product should ideally function as a cell factory, working directly on the spot where the target organism is harmful. Thus, understanding the mode of action is a crucial step in the assessment process.

Micro-organisms may produce a range of different metabolites and toxins (e.g. bacterial toxins or mycotoxins) which may have toxicological significance. The relevance of these metabolites to humans and non-target organisms should be assessed by using information from: toxicity and ecotoxicity studies; biological properties of the micro-organism; relationship of the micro-organism to known plant, animal or human pathogens; and mode of action. If on the basis of this information, metabolites are considered as being relevant, the potential exposure to these relevant metabolites should be assessed, in order to decide on their risk.

During the Ctgb workshop held in November 2015<sup>2</sup> secondary metabolites were specifically addressed. Secondary metabolites may be formed which are not necessarily involved in normal growth and viability. Secondary metabolites include toxins, antibiotics, and other compounds that may enhance the growth or survival of micro-organisms in a competitive environment. These metabolites should be regarded as a concern if literature indicates that a relevant metabolite can be formed based on information on a (related) species<sup>3</sup>. It should be considered if these metabolites are present in the product and/or generated during use. When limited information is available it may be necessary to perform an acute toxicity test, provide information on the presence of metabolites during production, and search for any available literature for

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<sup>2</sup> Workshop on the toxicological risk assessment of pesticides using micro-organisms, 13 November 2015. Board for the Authorisation of Plant Protection Products and Biocides.

<sup>3</sup> E.g. the mycotoxin-producing fungi are considered to be a relevant group for their production of secondary metabolites.

these metabolites. In case information is not sufficient a repeated dose toxicity study may be needed. The production of certain metabolites (e.g. phytotoxins), may intentionally be enhanced during fermentation, in this case the secondary metabolite would require further investigations.

In this Guidance secondary and primary metabolites are not addressed separately.

For micro-organisms standardised test methodology in general is scarce; there is a set of specific test guidelines by the US EPA Office of Prevention, Pesticides and Toxic Substances Chemical Safety and Pollution Prevention, the OPPTS 885 series. Other internationally recognized methods may be available, for example, there are ISO methods that may be applicable. In case non-standardised tests are available the quality of the methods need to be reported in detail and assessed for example, as regards relevance, representativeness, sensitivity, specificity, reproducibility, interlaboratory validation and predictiveness. For validation of chemical methods, an OECD guide exists (OECD No34; ENV/JM/MONO(2005)14.) and methods with microbials can be validated (this is possible for qualitative identification, while quantitative validation is more difficult and more variable than for chemicals). Although the OECD (chemical) Guidance Document for these analytical methods (Technical Material and Preparations; SANCO/3030/99 rev.4; 11/07/00) is not directly applicable, some of the principles can also be used for microbials. Validation of the methods, including the standard inter- and intra-laboratory testing (i.e. different laboratories applying the same protocol having the same result, and multiple replication of the protocol in the same laboratory giving the same result, within the confines of the applied and appropriate analytical method) is highly recommended.

A microbial biocidal product can be formulated in different ways in order to be most efficient. Biological properties, formulation and method of application related to exposure must be taken into account when assessing exposure and possible risk. Member States must take into account the fact that any co-formulants and the form in which the biocidal product is marketed may have an impact on the characteristics of the biocidal product compared to the active micro-organism.

A microbial biocidal product may contain micro-organisms and non-viable microbiological entities and co-formulants. It may also contain relevant metabolites/toxins produced during growth, residues from the growth medium, and microbial contaminants. Information on test conditions like the micro-organism, relevant metabolites/toxins and the biocidal product with residual growth medium and microbial contaminants is necessary for the assessment. A risk assessment on the active micro-organism(s), relevant metabolites/toxins, residual growth medium, co-formulants and microbial contaminants present in the biocidal product should always be carried out. The risk assessment should cover the proposed normal use of the biocidal product, together with a realistic worst case scenario including any relevant production and disposal issue. In evaluating applications and granting authorisations, it is necessary to consider the proposed practical conditions of use and in particular the purpose of use, the dose, the manner, frequency and timing of applications, the type of application in relation to exposure and the nature and composition of the biocidal product.

The detailed quantitative and qualitative information provided on the composition of the biocidal product needs to be provided, such as that concerning the active micro-organism (see above), relevant metabolites/toxins, residual growth medium, co-formulants and microbial contaminants present.

The results derived from the assessment of the exposure to the active micro-organism(s), relevant metabolites/toxins, residual growth medium, co-formulants and microbial contaminants should be integrated to produce an overall risk assessment for the biocidal product. Where quantitative results are not available the results of the qualitative assessments should be integrated in a similar manner. Ultimately the decision

about the type of assessment to be undertaken (quantitative, semi-qualitative or qualitative) depends on case-by-case considerations taking into account the available information and tools.

The risk assessment should determine:

- a) the hazards due to the biological properties of the micro-organism as well as physico-chemical properties of the formulation including co-formulants;
- b) the exposure to human, animals and to the environment;
- c) the risk to humans and animals;
- d) the risk to the environment; and
- e) the measures necessary to protect humans, animals and the environment from exposure, both during the proposed normal use of the biocidal product and in a realistic worst case situation.

The identification and assessment of potential adverse effects on human and animal health and the environment have to be scientifically based and performed on a case-by-case basis until further experience is reached.

For a biocidal product containing more than one active micro-organism or substances of concern, any adverse effects should also be considered together to produce an overall assessment for the biocidal product itself.

For genetically modified micro-organisms, Directive 2001/18/EC<sup>4</sup> of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms, must be taken into account and authorization must not be granted unless written consent, as referred to in Article 19 of Directive 2001/18/EC, has been granted for it. The evaluation completed in the framework of that Directive must be provided and taken into account.

## 1.2 Structure of the guidance

This document is based on different experiences listed below:

- Technical Notes for Guidance on data requirements for micro-organisms including viruses and fungi [[European Commission 2005, \(2013-12-10\)](#)]
- Experiences in product authorisations of microbial biocidal products containing *Bacillus thuringiensis* sub-species *israelensis* as well as microbial plant protection products.
- The Uniform Principles for micro-organisms of the Commission Regulation 546/2011<sup>5</sup> on plant protection products<sup>6</sup>.
- Discussions at the OECD/EU/KemI workshop on microorganisms June 2013.
- Discussions at the CTGB workshop on the toxicological risk assessment of pesticides using micro-organisms on November 2015<sup>7</sup>.

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<sup>4</sup> Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC.

<sup>5</sup> Commission Regulation (EU) No 546/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards uniform principles for evaluation and authorisation of plant protection products

<sup>6</sup> Note that the Uniform Principles for microorganisms on PPP are under revision and should be relied upon with caution.

<sup>7</sup> Final report available at <http://www.ctgb.nl/en/news/news-items/2015/11/13/workshop-on-the-toxicological-risk-assessment-of-pesticides-using-microorganisms-successful>.

The guidance consists of four key sections:

- (i) Identity, biological and technical properties/analysis;
- (ii) Effectiveness against target organism;
- (iii) Effects on human and animal health;
- (iv) Effects on non-target organisms and effects on the environment.

Each of these sections is separated into three parts:

- Part A Information requirements;
- Part B Hazard and risk assessment; and
- Part C Evaluation/conclusion/decision criteria.

The structure of the parts on information requirements follows the BPR Annex structure:

- a. The core data set (CDS) and additional data set (ADS) are listed in the same chapter.
- b. The specific rules for adaptation from standard information requirements (including those given by BPR Annex II and III column 3) are included in the respective endpoint sections, where available.

Headings and numbering of the requirements in sections relating to Information Requirements correspond to the legal text of the numbering in the BPR Annexes II and III. These headings are in ***italic green font*** to distinguish them from the section numbers of the Guidance document. These sections have a "⚠ NOTE to the reader" to highlight this.

This guidance concerns primarily micro-organisms consisting of bacteria, fungi or virus but can be extrapolated to any other micro-organisms. It should be seen as a first implementation of the principles in Annex VI of the Biocidal Products Regulation (BPR) for micro-organisms. The Guidance is not applicable to UVCB substances. With further practical experience gained, the document will need to be revised, taking into consideration the latest scientific information.

### 1.3 Audience of the guidance

This guidance is addressed to both prospective applicants intending to prepare a dossier on active micro-organisms and competent authorities who are required to evaluate the dossier.

Readers will find in part A of each section a description of the information requirements listed per endpoint as required by Annex II and Annex III of the BPR. The endpoints are divided into four sections according to their relevance to the four key sections (listed above (i) to (iv)).

Parts B and C of each section provide guidance to both applicant and evaluating competent authority on how the information listed in each section is evaluated and assessed and conclusions drawn.

#### NOTES to the reader:

1. Text written in *italics* originates from the BPR or its Annexes.
2. ECHA Guidance documents are given by published name and in italics, e.g. *Guidance on the compilation of safety data sheets*.
3. Link to ECHA Guidance documents on biocides webpage:

<http://echa.europa.eu/web/guest/guidance-documents/guidance-on-biocides-legislation>

4. Link to the ECHA Guidance documents on REACH webpage:  
<http://echa.europa.eu/web/guest/guidance-documents/guidance-on-reach>

## 2. Terms and Definitions

Some definitions in Article 3 of the BPR may lead to some confusion when dealing with micro-organisms and are therefore explained further below.

**Table 1 Article 3 Definitions**

Definition in the BPR	Explanation for applicability for micro-organisms:
<p><i>Art. 3 1 (b) Micro-organism means any microbiological entity cellular or non-cellular, capable of replication or of transferring genetic material, including lower fungi, viruses, bacteria, yeasts, moulds, algae, protozoa and microscopic parasitic helminths</i></p>	<p>Micro-organism is defined in Art. 3 1 (b). Pursuant to that definition only “viable microbiological entities”, i.e. microbiological entities capable of replication or of transferring genetic material, are considered as “micro-organisms” within the meaning of Article 3 1 (b) of the BPR.</p> <p>The present guidance does not cover “non-viable microbiological entities”, i.e. microbiological entities not capable of replication or of transferring genetic material, which thus do not fulfil the definition of “micro-organism” under the BPR.</p>
<p><i>Art. 3 1 (c) Active substance means a substance or a micro-organism that has an action on or against harmful organisms.</i></p>	<p>Active micro-organism</p> <p>Micro-organism is defined in Art. 3 1 (b) (see above). To distinguish between chemicals and micro-organisms the term active micro-organism is preferred rather than active substance.</p> <p>The term active micro-organism is also used in cases where microbiological entities are an integral part of the active substance, but where the biocidal effect is actually exerted through extracellular substances, e.g. proteinaceous toxins produced by these microbiological entities.</p>
<p><i>Art. 3 1 (f) Substance of concern means any substance, other than the active substance, which has an inherent capacity to cause an adverse effect, immediately or in the more distant future, on humans, in particular vulnerable groups, animals or the environment and is present or is produced in a biocidal product in sufficient concentration to present risks of such an effect.</i></p>	<p>Substance of concern has the same meaning as defined in Art. 3 1 (f). This does not concern the active micro-organism, but concerns any other substances in the formulation. A relevant metabolite/toxin could fall under the definition of substance of concern, but due to the association between active micro-organism and its metabolites/toxins, such substances could be regarded either as property/product of the active micro-organism or as a separate SoC.</p>



Definition in the BPR	Explanation for applicability for micro-organisms:
<p>Art. 3 1 (g) Harmful organism <i>means an organism, including pathogenic agents, which has an unwanted presence or a detrimental effect on humans, their activities or the products they use or produce, on animals or the environment.</i></p>	<p>Harmful organism has the same meaning as defined in Art. 3 1 (g). Pathogenic agents are included in that definition. A pathogenic agent has the potential ability to produce adverse health effects by using virulence factors with specific modes of action for entry and colonization. The active micro-organism should not be pathogenic to humans or animals, however pathogenicity could be a mode of action against harmful organisms.</p>
<p>Art. 3 1 (h) Residue <i>means a substance present in or on products of plant or animal origin, water resources, drinking water, food, feed or elsewhere in the environment and resulting from the use of a biocidal product, including such a substance's metabolites, breakdown or reaction products.</i></p>	<p>Residue has the same meaning as in Art. 3 1 (h). For biocidal products containing micro-organisms residues mean viable micro-organisms, substances produced in significant quantities by these micro-organisms or culture residues which persist after the disappearance of the micro-organisms and are of concern for human or animal health and/or the environment.</p>

**Table 2 Definitions and Explanations of microbiological terms**

Term	Definition
Antibiosis	<p>A relationship between two or more species in which one species is actively harmed (as by the production of toxins by the harming species). Antibiosis can also be an antagonistic association between an organism and the metabolic substances produced by another. Examples of antibiosis include the relationship between antibiotics and bacteria.</p>
Antigenic	<p>Any substance that, as a result of coming in to contact with appropriate cells, induces a state of sensitivity and/or immune responsiveness after a latent period (days to weeks) and which reacts in a demonstrable way with antibodies and/or immune cells of the sensitised subject <i>in vivo</i> or <i>in vitro</i>.</p>

Term	Definition
Antimicrobial	<p>Antimicrobial agents or antimicrobial(s) refer to naturally occurring, semi-synthetic or synthetic substances that exhibit antimicrobial activity (kill or inhibit the growth of micro-organisms, <i>i.e.</i> could be either microbiocidal or microbiostatic).</p> <p>The term 'Antimicrobial(s)' includes:</p> <ul style="list-style-type: none"> <li>• antibacterials/antibiotics, which refers to substances that are active against bacteria;</li> <li>• anticoccidials, which refer to substances that are active against coccidia, single cell protozoan parasites;</li> <li>• antifungals, which refer to substances that are active against fungi;</li> <li>• antimicrobial, which refers to substances that are active against micro-organisms;</li> <li>• antivirals, which refer to substances that are active against viruses, and</li> <li>• disinfectants, which refer to substances that are within the definition of product types 1, 2, 3, 4 and 5, as laid down in Annex V.</li> </ul>
Biopotency	Measure of the ability of a material to have a specified biochemical function.
CFU	Colony-forming unit; one or more cells that grow to form a single visible colony.
Clearance	Elimination of micro-organisms from a human or an animal, including elimination of the colonisation by pathogens of infected tissues.
Co-formulant	Any substance other than the active ingredient that is intentionally added to a biocidal product.
Colonisation	Establishment, proliferation and persistence of a micro-organism in an environment, such as on external (skin) or internal body surfaces (intestine, lungs) without tissue invasion or damage. For colonisation, the micro-organism should at least persist for a longer period than expected in a specific organ. The population of micro-organisms may decline but at a slower rate than normal clearance; it may be a steady population or it may be a growing population. Colonisation can be related to harmless and functional micro-organisms as well as to pathogenic micro-organisms. The possible occurrence of effects is not indicated.
Contaminant	An unwanted micro-organism.
Ecological niche	Unique environmental position occupied by a particular species, perceived in terms of actual physical space occupied and function performed within the community or ecosystem.

Term	Definition
Extra chromosomal genetic elements	Any additional genetic element besides the chromosomal DNA in prokaryotes and eukaryotes. In prokaryotes extra-chromosomal genetic elements are for example, plasmids, transposable elements or bacteriophage DNA. In eukaryotes the DNA of organelles such as mitochondria and chloroplasts, the plasmids of yeasts, transposable elements or virus DNA represent extra-chromosomal genetic elements. Extra-chromosomal genetic elements normally carry additional genetic information that can be beneficial, harmful or neutral to its carrier.
Host	An animal (including humans) plant or organism that harbours or nourishes another organism (parasite).
Host specificity	The range of different host-species that can be colonised by a microbial species or strain. A host-specific micro-organism colonises or has adverse effects on one or only a small number of different host-species. A non-host-specific micro-organism may colonise or may have adverse effects on a broad range of different host-species.
Impurity	The definition in the <i>Guidance for identification and naming of substances under REACH and CLP</i> used also for chemicals under the BPR applies: "An impurity is an unintended constituent present in a substance as manufactured. It may originate from the starting materials or be the result of the manufacturing process. While it is present in the final substance it was not intentionally added."
Infection	The introduction or entry of a pathogenic micro-organism into a susceptible host, whether or not it causes pathological effects or disease. The organism must enter the body of the host, usually the cells, and be able to grow to form new infective units. Simply ingesting a pathogen does not necessarily imply infection.
Infective	Capable of transmitting/causing an infection.
Infectivity/infectiveness:	The characteristics of a micro-organism that allow it to infect a susceptible host.
International Unit (IU)	Quantity of a substance that produces a specified effect when tested according to an internationally accepted biological procedure. Used (e.g.) for vitamins, hormones and similar biologically active substances.
International Toxic Unit (ITU)	Quantity of a toxin that produces a specified effect when tested according to an internationally accepted biological procedure.
Invasion	The entry of a micro-organism into the host body (e.g. actual penetration of the integument, gut epithelial cells, etc.). "Primary invasiveness" is a property of pathogenic micro-organisms.

Term	Definition
Metabolites	<p>Products resulting from degradative and biosynthetic reactions taking place within the micro-organism.</p> <ul style="list-style-type: none"> <li>• Primary metabolites: directly involved in normal growth, development and reproduction of a micro-organism. These metabolites are usually essential for survival under normal growth situations.</li> <li>• Secondary metabolites: not necessarily involved in normal growth and viability. Secondary metabolites include toxins, antibiotics, and other compounds that may enhance the growth or survival of micro-organisms in a competitive environment.</li> </ul>
Multiplication	Ability of a micro-organism to divide and grow during an infection.
Mycotoxin	A fungal toxin.
Non-viable micro-organism	A micro-organism that is not capable of replication or of transferring genetic material.
Non-viable residue	A residue that is not capable of replication or of transferring genetic material.
Parasitism	The relation between two or more organisms in which at least one organism benefits by causing harm to at least one of the other organism(s).
Pathogenicity	The ability of a micro-organism to cause disease and/or inflict damage on the host after infection and depends on host resistance or susceptibility. Many pathogens cause disease by a combination of (i) toxicity and invasiveness or (ii) toxicity and colonising ability. However, some invasive pathogens cause disease that results from an abnormal reaction of the host's defence system. Opportunistic pathogens exhibit pathogenicity usually only in susceptible individuals such as immunocompromised people. Facultative pathogens are pathogenic in healthy hosts, but can survive and/or grow outside their hosts cells. Obligate pathogens are incapable of survival and replication outside of their hosts.
Performance	Performance of a microbial biocidal product is the complex of activities related to the active substance and co-formulants, which include efficacy, efficiency, persistence in the receiving environment, toxicity, resistance to biotic and abiotic stresses. The term 'performance standard' in the TNsG for products evaluation (BPD TNsG; Chapter 7.4....) refers to the pre-determined efficacy that is required by Regulatory Authorities for authorisation of a biocidal product for a particular use. The term is synonymous with 'pass/fail' criteria and 'acceptability criteria'.
Persistence	The ability of a micro-organism to remain in a particular setting, e.g. the host, for a period of time after it is introduced.


Term	Definition
Proliferation	The ability of a micro-organism to reproduce and increase in numbers.
Relevant impurity	The definition in the <i>BPR Guidance on information requirements (Part A in Volumes I-IV)</i> applies: "An impurity can be considered of toxicological and/or ecotoxicological relevance. An impurity may be relevant even if it occurs in a quantity < 1 g/kg (e.g. very toxic substances like dioxin). Relevant impurities can be defined as constituents, including but not limited to, that meet the criteria to be classified as hazardous in accordance with Regulation 1272/2008 (CLP), or the available information (e.g. from (Q)SARs) indicates that the impurity has an (eco)toxicological hazard. Relevant impurities have the inherent capacity to cause unacceptable effects within the meaning of Article 19(1)(b) of the BPR. Compared to the active substance, relevant impurities show additional (or more severe) toxic properties (in the sense of the definition above).".
Relevant metabolite	Metabolites that form a major part of the mode of action and/or are present in significant amounts and/or produce an adverse effect on humans or the environment under practical conditions of use.
Relevant residue	Residues are considered relevant if a maximum residue level (MRL) or a waiting or re-entry safety period or other such precaution is required.
Symbiosis	A type of interaction between organisms where one organism lives in intimate association with another, which is favourable for both organisms.
TGAI	Technical Grade Active Ingredient - an active micro-organism and any associated metabolites/toxins, fermentation residues and contaminants as manufactured.
Toxicity	The injury or damage in a host caused by a poison or toxin where infection by and/or replication or viability of the micro-organism are not necessarily required (OECD Series on Pesticides No 67).
Toxins	Harmful substances, endo- or exotoxins, produced by micro-organisms. Microbial toxins are important virulence determinants responsible for pathogenicity and/or evasion of the host immune response. Compare metabolites, especially secondary metabolites above.
UVCB	Substances of Unknown or Variable composition, Complex reaction products or Biological materials.
Viable micro-organism	A micro-organism that is capable of replication or of transferring genetic material.
Viable residue	A residue that is capable of replication and/or of transferring genetic material.

<b>Term</b>	<b>Definition</b>
Viroid	Any of a class of infectious agents consisting of a small strand of RNA not associated with any protein. The RNA does not code for proteins and is not translated; it is replicated by host cell enzymes. Viroids are known to cause severe plant diseases.
Virulence	Measurement of the degree of disease producing ability of a micro-organism as indicated by the severity of the disease produced. Measure of the dosage (inoculum size) required to cause a specific degree of pathogenicity. It is measured experimentally by the median lethal dose (LD <sub>50</sub> ) or median infective dose (ID <sub>50</sub> ) in an adequate unit, usually given in CFU.

## 3. Identity, biological and technical properties/analytics

### 3.1 Part A: Information requirements

#### 3.1.1 Active Micro-organism

 **NOTE to the reader:** The endpoints described below are numbered in accordance with the BPR, Annex II Information Requirements for Active Substances, Title 2 Micro-organisms, Core data set and additional data set for active substances.

Headings are shown in *italic green font* to distinguish them from the general section numbers of the Guidance document.

#### *1. Applicant*

##### *1.1. Name and address*

- Name and address of the natural or legal entity of the applicant.

##### *1.2. Contact person*

- Names, address, telephone and fax numbers, email, and other contact information of the applicant.

##### *1.3. Manufacturer (name, address and location of manufacturing plant)*

- Name and address of the manufacturer(s);
- Name, address and location of manufacturing plant(s).

If the applicant is supplied by several manufacturers all of them need to be listed and the identity data of each manufacturer needs to be provided.

#### *2. Identity of the micro-organism*

The taxonomic identification of a micro-organism is one of the key elements in any risk assessment.

Methods to generate characterisation data range from traditional culture-based phenotypic and biochemical tests to molecular techniques.

##### *2.1. Common name of the micro-organism (including alternative and superseded names)*

Common name or alternative and superseded names and code names used during the development, if any, must be provided.

##### *2.2. Taxonomic name and strain*

Each micro-organism that is the subject of the application should be identified and named at the strain level. The scientific name and taxonomic grouping, (i.e. family, genus, species, strain, serotype, pathovar or any other denomination relevant to the micro-organism), must be stated. For bacteria the Guidance Document on the Use of Taxonomy in Risk Assessment of micro-organisms applies: Bacteria, OECD Environment, Health and Safety Publications, Series on Harmonisation of Regulatory Oversight in Biotechnology, No.29, Paris 2003.

It must be indicated whether the micro-organism:

- is a wild type;
- is a spontaneous or induced mutant;
- has been modified, using techniques described in Annex I, Part 2, and Annex IB to Directive 2001/18/EC.

In the last two points (above), all known differences between the modified micro-organism and the parent wild strain must be provided. Detailed information/protocols should be provided on the genetic modification, the production and the isolation of the mutant strains.

### **2.3. Collection and culture reference number where the culture is deposited**

The micro-organism must be deposited at an internationally recognised culture collection. The accession number given and other details, including details of country and source of isolation, must be submitted.

### **2.4. Methods, procedures and criteria used to establish the presence and identity of the micro-organism**

The most appropriate justified technology should be used to identify and characterise the micro-organism at the strain level. The appropriate test procedures and criteria used for identification (e.g. morphology, biochemistry, serology and molecular identification) must be provided. For bacteria two Guidance Documents apply:

1. Guidance Document on Methods for Detection of Micro-organisms introduced into the Environment: Bacteria, OECD Environment, Health and Safety Publications Series on Harmonisation of Regulatory Oversight in Biotechnology No. 30, Paris 2004;
2. Guidance Document on the Use of Taxonomy in Risk Assessment of micro-organisms: Bacteria, OECD Environment, Health and Safety Publications, Series on Harmonisation of Regulatory Oversight in Biotechnology, No.29, Paris 2003.

Several methods could be used to provide information about the identity of the micro-organism. Preferably a combination of both phenotypic and genotypic methods should be used. Scientific justification should be provided when genotypic methods are not used.

#### 1. Phenotypic methods:

- morphological methods (e.g. colony shape, cell stain, light and electron microscopy);
- physiological methods (e.g. growth temperature, pH of growth range, oxygen and carbon dioxide tolerance, salt tolerance);
- metabolic methods (e.g. nutritional profiles based on utilisation and/or degradation, enzymatic activity).

#### 2. Chemotaxonomic methods:

- Typing (e.g. protein, lipopolysaccharide and fatty acid profiles and serotyping in particular serology profiling).

#### 3. Genotypic methods:

- Full genome sequencing;
- Sequencing of regions may generally be accepted for species/strain identity, e.g. 16s rDNA for bacteria; ITS region for fungi; housekeeping genes (in particular taxon specific DNA/RNA sequences);
- genotypic methods (e.g. gene probing);
- DNA base ratios and DNA hybridization;
- DNA-based typing methods (e.g. DNA fingerprinting);
- RNA-based typing methods (e.g. RNA fingerprinting).



The amount of analyses to be performed for identification will vary depending on the specific micro-organism in question. As an example, the identification of *Bacillus* strains may be more cumbersome with respect to other micro-organism due to the tight relatedness of species within this genus.

Most likely, an individually tailored subset of the above mentioned techniques will be the best way to safely and efficiently address the identity of a micro-organism in question. Therefore, the necessary experimental procedures should be carefully designed based on case-relevant scientific, peer-reviewed literature. For example, phenotypic profiling methods may be useful for identification of certain micro-organisms but may fail in case of others, for example, due to low resolution. Sufficient genetic data should also be generated in order to deduce phylogenetic trees.

While a 16s rRNA sequence alone may be sufficient to address systematics of some micro-organisms, sequencing multiple genes may be necessary for phylogenetic differentiation of others that cannot be distinguished solely based on 16s rRNA sequence.

### **2.5. Specification of the technical grade active ingredient**

This section should include the reference specification of the technical grade active substance (TGAI). The information on the identity of the active micro-organism, metabolites/toxins relevant for the biocidal effect, and other chemical constituents or contaminants as manufactured such as additives must be provided in 2.6-2.9.

### **2.6. Method of production and quality control**

Full information on how the micro-organism is produced in bulk must be provided.

The production method/process must be subject to a continuous quality control by the manufacturer. In particular, the occurrence of spontaneous changing of major characteristics of the micro-organism and of the absence/presence of contaminants should be monitored. The quality assurance criteria for the production should be submitted.

The techniques used to ensure a uniform product and the assay methods for its standardisation, maintenance and purity of the micro-organism must be described and specified (e.g. HACCP).

If metabolites/toxins are responsible for the mode of action, the method(s) used for identification and quantification of these metabolites/toxins must be stated (e.g. SDS-PAGE, ELISA, Bradford assay, Western Blot). Where biopotency is used to characterize the biocidal effect, the applied bioassay must be described in detail, including information about the standard (source, storage conditions, type), the test-organism (type, density, development stage), and test conditions (temperature, nutrient supply, light). Biopotency can be expressed e.g. in ITU (International Toxic Units).

Where the information provided relates to a pilot plant production system, the information required must again be provided once industrial scale production methods and procedures have been stabilised.

### **2.7. Content of the micro-organism**

The minimum content of the active micro-organism(s) in the technical grade active ingredient (TGAI) has to be submitted. The maximum content needs to be reported when concern exists of a risk to human health or environment due to exposure to the microbe, or if secondary toxins/metabolites are produced. The contents should be expressed in appropriate terms taking into account the hazard profile and mode of action (the number of viable active units (e.g. cfu) per volume and per weight as well as any other manner that is relevant to the active micro-organism) (e.g. international toxic units ITU or biopotency in international units IU) within this provision.

Once production changes from a pilot plant production system to an industrial scale, an application for technical equivalence assessment has to be filed to ECHA.

Methods for the quantification of active microorganisms should follow recognised international standards, or validated internal methods. Method validation should include showing specificity with appropriate positive and negative controls<sup>8</sup> and repeatability by performing five independent measurements.

### **2.8. Identity and content of impurities, additives, contaminating micro-organisms**

It is desirable to have a TGAI without contaminating micro-organisms. The level and nature of acceptable contaminating microorganisms should be judged from a risk assessment point of view.

Relevant metabolites/toxins known to be formed by the micro-organism (see endpoint 3.5 for the active micro-organism) should be identified and characterized in the TGAI. For products that are produced in a continued process this may not be possible for the TGAI and data on the product could then be used instead.

Where relevant, detailed information on all constituents such as condensates, culture medium, etc., must be provided. In the case of chemical impurities or additives that are relevant for human and animal health and/or the environment, the identity and maximum content, must be provided. The information on the identity of the chemical constituents such as additives must be provided as outlined in the BPR Annex II Title I, endpoint 2.10.

The contents of the chemical constituents should be expressed in terms as provided for in Annex VI to the CLP Regulation<sup>9</sup> and appropriate terms should be used for micro-organisms (number of active units per volume or weight or any other manner that is relevant to the micro-organism).

### **2.9. Analytical profile of batches**

Representative 5-batches of the TGAI (technical grade active ingredient), i.e. preferably 5 full-scale production batches, must be analysed for content of active microorganisms, impurities, additives, and contaminating micro-organisms, as appropriate. The analytical results must include quantitative data, in appropriate terms (e.g. g/kg for chemical constituents, and colony forming units per weight (g) or volume (L) or another appropriate term for micro-organisms and/or metabolites/toxins if relevant for the biocidal effect). Batches from lab-scale or pilot production systems can be provided if no full-scale production batches are available yet. Once production changes from a pilot plant production system to an industrial scale, an application for technical equivalence assessment has to be filed to ECHA.

In the case where the TGAI is not stable, because it is produced and formulated in an integrated process, the 5-batch analysis should be conducted with the end use product. Furthermore if alternative processing is used in some step of the manufacturing process, 5 batches should be analysed for each different processing method in order to demonstrate the equivalency. In a continuous process, an analytical profile of the product is required, whereas in a discontinuous process the profile must be performed on the TGAI.

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<sup>8</sup> Positive control: a culture of the micro-organism to be determined; negative control(s): blank samples without the micro-organism.

<sup>9</sup> Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.

### **3. Biological properties of the micro-organism**

Due to their metabolic versatility, micro-organisms are present everywhere in the earth's biosphere, playing many vital functions in environment. Microorganisms are introduced or augmented in the local environment. The biological properties of the microorganisms are key elements to be considered in the risk assessment.

#### **3.1. General information on the micro-organism**

Familiarity, interpreted as the availability of relevant knowledge of the micro-organism, should be presented. Where available, appropriate literature should be cited. However, when referring to data available in the open literature, the reliability and the relevance of the data for the assessment should be discussed. Moreover, a full reference list (including authors, title, journal, year, number and pages) should be annexed to the competent authority report. While this actually applies to almost every section of the present guidance it is emphasized here because the information to be reported under section 3 of Annex II Title 2 of the BPR will rely mostly, if not entirely on literature and not on experimental data.

##### **3.1.1. Historical background**

Information on the historical background of the wild type micro-organism should be submitted.

##### **3.1.2. Historical uses**

Information on historical uses (tests/research projects or commercial uses) should be submitted.

##### **3.1.3. Origin, natural occurrence and geographical distribution**

The geographical region and the place in the ecosystem (e.g. host plant, host organism, or soil) from which the micro-organism was isolated first must be stated. The method of isolation of the micro-organism should be reported. The natural level of abundance of the micro-organism is required at a species level for all compartments directly or indirectly exposed.

In the case of a mutant, or a genetically modified micro-organism (as defined in Annex IA of the Directive 2001/18/EC, and Annex IB to Directive 2001/18/EC), detailed information should be provided on its production and isolation and on the means by which it can be clearly distinguished from the parent wild strain.

#### **3.2. Development stages/life cycle of the micro-organism**

Information on the life cycle of the micro-organism, described symbiosis, parasitism, competitors, predators, etc., including host organisms, as well as vectors for viruses, must be presented. Eventually, symbiosis, parasitism, virulence and other issues are usually covered in sections on human, animal and plant health. If this is the case and in order to avoid redundant information, it is sufficient to make reference to these sections. The type of reproduction of the micro-organism must be stated.

Information on the occurrence of resting stages and their survival time, their potential to transform into virulent stages and their infection potential must be provided along with any information on the biological cycle in the relevant environment (e.g. soil, water). This information should include, where relevant, a discussion on the resistance of a given resting stage against environmental conditions (heat, drought, salt, UV-radiation etc.) as well as conditions/circumstances which induce or promote germination; (see also 3.7 (below) and make cross references to overlapping information).

In cases where information on life cycle is mainly retrieved from scientific literature and/or where reference can be made to dedicated in-depth literature, appropriate citations are requested.

Competitors and predators may adversely interfere with field applications of the biocidal micro-organism and should be given attention from this point of view.

### **3.3. Relationships to known plant or animal or human pathogens**

The possible existence of one or more species of the genus of the active and/or, where relevant, contaminating micro-organisms known to be pathogenic to humans, animals, plants or other non-target species and the type of disease caused by them must be indicated. It must be stated whether it is possible, and if so, by which means to clearly distinguish the active micro-organism from the pathogenic species. When appropriate, particularly with regard to detection techniques, reference can be made to sections on identification and quality control. Appropriate scientific literature on related pathogens should be cited.

### **3.4. Genetic stability and factors affecting it**

If the micro-organism contains plasmids or other mobile genetic elements known to be involved in biocidal activity, pathogenicity, toxicity, resistance etc., these must be identified. Measures taken to minimise genetic drift should be described, for example, length of the fermentation process, *in vitro* or *in vivo* propagation, inoculation from the original source.

### **3.5. Information on the production of metabolites (especially toxins)**

Some micro-organisms may secrete a wide range of metabolites, mostly products of secondary metabolism as a result of growth or as a response to environmental conditions in order to regulate their own growth, control competitors or foster other organisms beneficial to them. These metabolites, including toxins, thus may be produced *in situ*, post-application and humans may be exposed, depending on the product type and the use pattern, if these end up in food or animal feed. These metabolites could vary in structure, some are simple organic molecules such as antimicrobial agents produced by fungi and some are peptides or proteins.

A complete identification and characterisation of all metabolites which are produced by microorganisms under different (environmental) conditions is currently not feasible for technical reasons. However, the potential for the micro-organism 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, taken together with that for one or more biocidal products containing the micro-organism, must be sufficient to permit an evaluation to be made as to the risk for man and/or environment, arising from potential exposure to the micro-organism and metabolites (toxins).

### **3.6 Production and resistance to antibiotics and other anti-microbial agents**

Many micro-organisms produce some antimicrobial metabolites. Interference with the use of these metabolites in human or veterinary medicine must be avoided at any stage of the development of a microbial biocidal product. The level of production of any known antibiotics used in human or veterinary medicine by the micro-organism must be indicated.

Information on the micro-organism's resistance or sensitivity to antibiotics or other antimicrobial agents must be provided. Information on the stability, in terms of genetic transfer, is of particular interest if these genes are carried on mobile genetic elements, since this may be of medical relevance.

### **3.7. Robustness to environmental factors**

Any particular sensitivity of the micro-organism and its life stages to certain environmental conditions (e.g. UV light, temperature, pH, humidity, nutrition requirements, etc.) for survival and growth of the micro-organism must be stated.

The temperature range at which the micro-organism grows must be determined, including minimum, maximum and optimum temperatures. This information is of particular value as a trigger for studies of effects on non-target organisms including humans but also for the efficacy of the active micro-organisms.

### **3.8. Further information on the micro-organism**

Any further relevant information on the micro-organisms must be provided, for example, if there is evidence of unintended effects on non-target materials, substances or products, information must be submitted.

## **4. Methods of detection and identification**

### **Introduction**

Post-approval monitoring may be considered for all areas of risk assessment. Analytical methods for monitoring are also required if a suitable residue definition is derived and maximum residue levels (MRLs) or other action levels are established. However, in order to be able to detect only the strain of interest, it is essential that the organism possesses at least one trait that can be used to distinguish it from all other micro-organisms. Therefore a non-indigenous strain can be more easily detected than a strain that is already present in the area of application. Reference is made to the Guidance Document on Methods for Detection of Micro-organisms introduced into the Environment: Bacteria, OECD Environment, (Health and Safety Publications Series on harmonisation of Regulatory Oversight in Biotechnology No. 30, Paris 2004) and to the Working Document On The Evaluation Of Microbials For Pest Control, chapter 1 (ENV/JM/MONO(2008)36).

The applicant has to provide a justification for the analytical method used for generation of data as required in the BPR or for other purposes.

Descriptions of methods must be provided and include details of equipment, materials and conditions used. The applicability of any internationally recognised method must be reported. As far as practicable these methods must employ the simplest approach, involve the minimum cost, and require commonly available equipment.

On request the following samples must be provided:

- samples of the micro-organism as manufactured; the TGAI may be a theoretical step in the production process of the biocidal product and in that case a sample of the biocidal product may be provided instead
- analytical standards of relevant metabolites (especially toxins) and all other constituents included in the residue definition;
- if available, samples of reference substances for the relevant impurities.

### **4.1. Analytical methods for the analysis of the micro-organism as manufactured**

The information on the methods for the analysis of the micro-organism as manufactured and chemical constituents such as additives is provided for example, under endpoints 2.4, 2.7 and 2.8 for the active micro-organism.

#### **4.2. Methods used for monitoring purposes to determine and quantify residues (viable or non-viable)**

Post registration monitoring methods to determine and quantify residues (viable or non-viable) of the micro-organism and relevant metabolites/toxins, on and/or in crops, in foodstuffs and feeding stuffs, in animal and human body tissues and fluids, in soil, in water (including drinking water, ground water and surface water), in sediment and in air should be submitted where relevant.

Analytical methods for amount or activity of proteinaceous products should also be included, for example, by testing exponential cultures and culture supernatants in an animal cell bioassay.

The information on characterisation and identity is provided in section 10 Measures necessary to protect humans, animals and the environment for active micro-organism (Annex II, Title 2, endpoint 10).

### **10. Measures necessary to protect humans, animals and the environment**

#### **10.1. Recommended methods and precautions concerning handling, use, storage, transport or fire**

A safety data sheet similar to that required for chemical active substances in the REACH Regulation must be provided for the TGAI, when applicable.

For further guidance see *Guidance on the BPR, Volume I, Part A, Section 12.6*.

#### **10.2. Emergency measures in case of an accident**

If relevant, information on procedures and methods used for rendering the micro-organism harmless in the environment (e.g. water or soil) must be provided.

#### **10.3. Procedures for destruction or decontamination**

Methods to dispose safely of the micro-organism, or where necessary to kill it prior to disposal, and methods to dispose of contaminated packaging and contaminated materials, must be fully described. Data must be provided for such methods to establish their effectiveness and safety. The methods must take into account the information submitted under endpoint 4 for active micro-organism.

#### **10.4. Procedures for waste management**

Procedures for destruction and decontamination must be developed for both small quantities (user level) and large quantities (warehouse level). The procedures must be consistent with provisions in place relating to the disposal of waste and of toxic waste (Directive 2008/98/EC, as last amended by Regulation (EC) No 1882/2003). The means of disposal proposed should be without unacceptable influence on the environment and be the most cost effective and practical means of disposal feasible. Information has to be submitted in regard to whether the presence of the micro-organisms in the waste makes it hazardous pursuant to Annex III to Directive 2008/98/EC, and in particular if it displays one of the hazard-properties listed in that Annex, in particular infectiousness (H9).

#### **10.5. Monitoring plan to be used for the active micro-organism including handling, storage, transport and use**

For a micro-organism that has adverse effects on humans, non-target organisms or the environment, a monitoring plan may be considered after the placing on the market of a biocidal product containing the micro-organism as active substances.

The objective of a monitoring plan is to confirm that any reference values set or any risk mitigation measures proposed do protect from adverse effects.

The interpretation of the data collected by monitoring should be considered in the light of other existing environmental conditions and activities. Where changes in the environment are observed, further assessment should be considered to establish whether the changes are due to the micro-organism released or its use, or if such changes may be the result of environmental factors other than the placing of the product on the market.

Experience and data gained through the monitoring of experimental releases of the micro-organism may assist in designing the post marketing monitoring regime required for the placing on the market of biocidal products containing micro-organisms as active substances.

The design of the monitoring plan should be detailed on a case by case basis taking into account the outcome of the risk assessment and also taking into account the characteristics of the micro-organism, the characteristics and scale of its intended use and the range of relevant environmental conditions where the micro-organism is expected to be released. Attention could be paid to the fact that most micro-organisms disappear in a relatively short time after application, mainly due to competition with indigenous micro-organisms.

## **11. Classification and labelling**

### **11.1. Relevant risk group specified in Article 2 of Directive 2000/54/EC**

A proposal for allocation of the micro-organism to one of the risk groups outlined in Article 2 of Directive 2000/54/EC<sup>10</sup> should be provided.

The provisions of the CLP Regulation cannot be used for the micro-organisms and thus they cannot be classified or labelled under the current classification and labelling system. However, the chemical constituents in a biocidal product, containing the micro-organisms, may trigger classification and labelling according to the CLP Regulation and other specific labelling requirements can apply (please refer to section 3.1.2 of this guidance, endpoint 12). The warning phrase "Micro-organisms may have the potential to provoke sensitising reactions" should always be applied to the active substance (micro-organism) and to the biocidal products except if there is scientific evidence that the active micro-organism has no sensitizing potential<sup>11</sup>.

### **3.1.2 Biocidal Product**



**NOTE to the reader:** The endpoints described below are numbered in accordance with the BPR, Annex III Information Requirements for Biocidal Products, Title 2 Micro-organisms, Core data set and additional data set for active substances.

Headings are shown in **italic green font** to distinguish them from the general section numbers of the Guidance document.

#### **1. Applicant**

##### **1.1. Name and address**

- Name and address of the natural or legal entity of the applicant and prospective authorisation holder, if different

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<sup>10</sup> Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work.

<sup>11</sup> CTGB workshop harmonization of the toxicological risk assessment for microorganisms used in plant protection (November 2015).

### 1.2. Contact person

- Names, address, telephone and fax numbers, email, and other contact information of the applicant and prospective authorisation holder, if different.
- The authorisation holder is required to have a permanent office with a legally responsible representative within the territory of the European Union.

### 1.3. Manufacturer and formulator of the biocidal product and the active substance(s) (names, addresses, including location of plant(s))

- Name, address and location of manufacturing plant(s).

## 2. Identity of the biocidal products

### 2.1. Trade name or proposed trade name


- If different trade names are used in different Member States, all of those have to be cited.

### 2.2 Manufacturer's development code and number of the product, if appropriate

- Company(ies) code number(s) or internal name(s).

### 2.3 Detailed quantitative (g/kg, g/l or % w/w (v/v)) and qualitative information on the constitution, composition and function of the biocidal product, e.g. micro-organism, active substance(s) and product co-formulants and any other relevant components.

Annex III, Title 2, 2.3 Column 1 states: *All relevant information on individual ingredients and the final composition of the biocidal product shall be given.*

 **Note to the Reader:** The title of the endpoint as quoted from the BPR is incorrect because it is mainly focussing on chemicals, for example, using g/kg when for the TGAI appropriate units for micro-organisms should be used.

All relevant information on individual ingredients and the final composition of the biocidal product must be given, including relevant information on the active and contaminating micro-organisms as well as toxins, if relevant for the mode of action, using appropriate units.

Each micro-organism that is the subject of the application should be identified and named at the strain level as set in Annex II, Title 2, Section 2 of the BPR. In addition, the development phase of the micro-organism (e.g. spores, mycelium) in the marketed product must be stated. In case the product contains only non-viable cells due to sterilization, information about the applied sterilization procedure must be provided.

For biocidal products the following information must be submitted:

- the content of the TGAI in the biocidal product. The information must include the minimum content of the viable material; maximum content is needed if risk to human health/environment is possible through the exposure to the microorganism, or if secondary toxins/metabolites are produced.
- the content of toxins or other metabolites relevant to the mode of action;
- the content of other formulants in the biocidal product,
- the content of all other relevant components and contaminating micro-organisms, derived from production process.

The contents should be expressed in terms as provided for in the CLP Regulation for chemicals and appropriate terms should be used for micro-organisms (number of active units per volume or weight or any other unit that is relevant to the micro-organism).



The identity and maximum content of key indicator contaminating micro-organisms, expressed in the appropriate unit, must be reported. Some guidance on contaminants is available in OECD Series on Pesticides No 65; to be noted, however, that contaminant limits for microbial pest control products cannot be applied directly as such to biocidal products) as outlined in Annex III, Title 2, Section 2 of the BPR.

Methods used for the detection and quantification of microorganisms should be internationally recognised standard methods (e.g. ISO method), or validated internal methods. For method validation specificity should be demonstrated with appropriate positive and negative controls<sup>12</sup> and repeatability by analysing five samples independently.

Chemicals (inert components, by-products, etc.) must be identified as outlined in Annex III, Title 1, Section 2, endpoint 2.3 of the BPR.

Where the information provided does not fully identify a component, such as condensate, culture medium, etc., detailed information on the composition must be provided for each such component.

#### **2.4. Formulation type and nature of the biocidal product**

A full description of the physical nature and state of the biocidal product must be provided, together with a proposal for a suitable description of the type of biocidal product and a proposal for its definition, for example, emulsifiable concentrate, wettable powder, solution, etc.

Further Guidance:

- Manual on development and use of FAO and WHO specifications for pesticides, second revision, (FAO, 2010).

### **3. Biological, physical, chemical and technical properties of the biocidal product**

The information must provide direct input parameters for assessing biological, physical, chemical and technical hazards, prerequisites for performing and guidance information for optimising other tests.

#### **3.1. Biological properties of the micro-organism in the biocidal product**

The product could display other biological properties than those of the active substance, the micro-organisms, by itself. In cases where the formulation has an impact on the expressed biological properties, information should be given.

#### **3.2. Appearance (at 20°C and 101.3 kPa)**

##### **3.2.1. Colour (at 20°C and 101.3 kPa)**

A description of the colour (range) , if any, and the physical state of the biocidal product, must be provided. If it is unlikely that the colour is influenced by minor temperature or pressure changes, as can be expected for microbial products, then the determination of the colour can take place at ambient conditions of the test facility.

##### **3.2.2. Odour (at 20°C and 101.3 kPa)**

A description of the odour may be provided be provided if available.

Testing for "Odour" is not compatible with QHSE procedures in most labs nowadays, because it asks for actively inhaling "chemical vapour". Therefore, it is recommended to omit this test.

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<sup>12</sup> Positive control: a culture of the microorganism to be determined (without other formulants); negative control(s): blank samples that could contain the other formulants, but without the cells of interest.

### 3.3. Acidity, alkalinity and pH value

Acidity, alkalinity and pH will be determined unless it can be justified that it is technically or scientifically not necessary to perform such studies. In accordance with the data requirements for chemicals in biocidal products, the acidity/alkalinity must be determined when the pH of the biocidal product at 1% dilution or dispersion is < 4 or >10. The acidity/alkalinity should be determined using CIPAC method MT 31. Alternatively, test according to CIPAC method MT 191.

Test according to the OECD Test Guideline 'Determination of pH, Acidity and Alkalinity' which is currently being developed.

### 3.4. Relative density

For liquids and solids, test according to EC method A.3 (Relative Density), based on OECD Test Guideline 109 (Density of Liquids and Solids), which was revised in October 2012.

Further Guidance:

- *Guidance on information requirements and chemical safety assessment Chapter R.7a<sup>13</sup>: Endpoint specific guidance, R.7.1.4 Relative Density,*

### 3.5. Storage stability, stability and shelf-life

While standard storage stability testing at elevated temperatures<sup>14</sup> may not be appropriate, consideration relating to the integrity of the product should be reflected in the label as supported by data. The following general advice should be considered when determining the shelf life:

- the duration of the storage stability test must cover the storage period specified on the product label;
- the content of microbial active ingredient/biopotency before and after storage stability test should not be below the minimum content given;
- if a storage temperature is stipulated on the product label (i.e. store refrigerated at 5°C), then the storage stability test should in general be conducted at this temperature; If no storage temperature is stipulated on the product label, the shelf life of the plant protection product at ambient temperature must be determined.
- the storage stability test should be normally conducted in the proposed product packaging (in specific situations different packaging size or material may be justified).

Further guidance:

- Within the BioPesticide Steering Group (BPSG) an issue paper on storage stability for products containing micro-organisms is being developed.
- OECD Series on Pesticides No 65 (or SANCO/12116/2012 rev.0) about the determination of microbial contaminants. It should be noted, however, that the contaminant limits for microbial pest control products cannot be applied directly as such to biocidal products.

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<sup>13</sup> The whole set of *Guidance on Information Requirement and Chemical Safety Assessment* under REAH is available at <http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment>.

<sup>14</sup> Outlined in Commission Regulation (EU) No 284/2013.

- “Manual on the Development and Use of FAO and WHO Specifications for Pesticides” for guidelines on the properties that need to be determined before and after storage.

### **3.5.1. Effects of light**

If it is known that the biocidal product is susceptible to light the following should be reported:

- what effect is likely to occur (e.g. accelerated degradation of the MPCA);
- what measurements are chosen to avoid an unwanted effect (e.g. use of a light proof packaging material).

### **3.5.2. Effects of temperature and humidity**

The physical and biological stability of the biocidal product at the recommended storage temperature including information on the growth of contaminating micro-organisms must be determined and reported. The conditions under which the test has been performed must be justified.

Where the type and composition of the formulation would allow growth or proliferation of potentially available microbial contaminants at the recommended conditions of storage, this has to be addressed.

Cold stability testing should be considered where cold storage may negatively affect the physical stability of the formulation. No negative impact is expected for the biological activity of micro-organisms in microbial formulations when stored around 0 °C. When storage stability for shelf-life is shown at low temperature, e.g. refrigeration, no additional cold stability testing is required at 0 °C.

The shelf life of the biocidal product at the recommended storage temperature must be reported. Where shelf life is less than two years, the shelf life in months, with appropriate temperature specifications, must be reported. Useful information could be found in GIFAP Monograph No 17.

### **3.5.3. Reactivity towards the container**

Information on the possible effects of the micro-organisms or its metabolic products on the material of the container must be provided. Recommendations for the material and storage of the container should be given.

### **3.5.4. Other factors affecting stability**

This endpoint is redundant. The issue of stability is already sufficiently addressed in data point 3.5.1 to 3.5.3. No further information is considered to be needed.

## **3.6. Technical characteristics of the biocidal product**

Testing of physical properties should follow the requirements and test methods described for chemical formulations. Where physical tests are not applicable to microbial formulations, this can be justified.

The *Guidance on information requirements* in Volume I Part A could be consulted but it is very important for products containing micro-organisms that the tests are performed at temperatures compatible with survival of the micro-organism.

Further relevant information:

- Within the BioPesticide Steering Group (BPSG) an issue paper on storage stability for products containing micro-organisms is being developed (expected 2<sup>nd</sup> half of 2016).

- OECD 65 (or SANCO/12116/2012 rev.0) for the determination of microbial contaminants.
- Manual on development and use of FAO and WHO specifications for pesticides (WHO, 2010).

### **3.6.1. Wettability**

The wettability of solid biocidal products which are diluted for use (e.g. wettable powders and water dispersible granules), must be determined and reported according to CIPAC Method MT 53.3.

### **3.6.2. Suspensibility and suspension stability**

The suspensibility of water dispersible products (e.g. wettable powders, water dispersible granules, suspension concentrates) must be determined and reported according to CIPAC Method MT 184.

The spontaneity of dispersion of water dispersible products (e.g. suspension concentrates and water dispersible granules) must be determined and reported according to CIPAC Methods MT 160 or MT 174 as appropriate.

### **3.6.3. Wet sieve analysis and dry sieve test**

In order to ensure that dustable powders have a suitable particle size distribution for ease of application, a dry sieve test must be conducted and reported according to CIPAC Method MT 59.1 or MT 170. In the case of water dispersible products, a wet sieve test must be conducted and reported according to CIPAC Method MT 185.

### **3.6.4. Emulsifiability, re-emulsifiability and emulsion stability**

The emulsifiability, emulsion stability and re-emulsifiability of biocidal products which form emulsions, must be determined and reported according to CIPAC Method MT 36.3.

The stability of dilute emulsions and of preparations which are emulsions, must be determined and reported according to CIPAC Method MT 20 or MT 173.

### **3.6.5. Particle size distribution content of dust/fines, attrition and friability**

The size distribution of particles in the case of powders, must be determined and reported according to OECD Method 110. The nominal size range of granules for direct application must be determined and reported in accordance with CIPAC MT 58.3, for water dispersible granules in accordance with CIPAC MT 170.

The dust content of granular biocidal products, must be determined and reported according to CIPAC Method MT 171. If relevant for operator exposure the particle size of dust must be determined and reported according to OECD Method 110.

The friability and attrition characteristics of granules, must be determined and reported once internationally agreed methods are available. Where already data are available they must be reported together with the method used.

### **3.6.6. Persistent foaming**

The persistence of foaming of preparations to be diluted with water must be determined and reported for example, according to CIPAC method MT 184.

### **3.6.7. Flowability / Pourability / Dustability**

- The flowability of granular preparations must be determined and reported e.g. according to CIPAC method MT 172.1.
- The pourability of suspensions must be determined and reported e.g. according to CIPAC method MT 148.

- The dustability of dustable powders must be determined and reported e.g. according to CIPAC method MT 34.

### **3.6.8. Burning rate - smoke generators**

Not relevant for products containing micro-organisms.

### **3.6.9. Burning completeness- smoke generators**

Not relevant for products containing micro-organisms.

### **3.6.10. Composition of smoke -smoke generators**

Not relevant for products containing micro-organisms.

### **3.6.11. Spraying pattern –aerosols**

Information on the spraying pattern of the biocidal product and the aerosol formation during its use must be reported in the context of the dispersal routes of the micro-organisms and its pathogenicity.

### **3.6.12. Other technical characteristics**

Any other relevant technical characteristic that is not covered by this Guidance should be reported here.

## **3.7. Physical, chemical and biological compatibility with other products including biocidal products with which its use is to be authorised or registered**

This is relevant to products and active ingredients with which the product will be used simultaneously.

### **3.7.1. Physical compatibility**

Possible physical incompatibility with any products or active ingredients should be mentioned.

### **3.7.2. Chemical compatibility**

Possible chemical incompatibility with any products or active ingredients should be mentioned.

### **3.7.3. Biological compatibility**

Possible biological incompatibility with any products or active ingredients should be mentioned.

## **3.8 Surface tension**

For all liquid biocidal products the surface tension at 1 g/l should be determined. For liquid biocidal products containing  $\geq 10\%$  hydrocarbons and for which the kinematic viscosity is less than  $7 \times 10^{-6}$  m<sup>2</sup>/sec at 40 °C the surface tension of the biocidal product as formulated should be determined at 25 °C.

Test according to EC method A.5 (Surface Tension) and OECD Test Guideline 115 (Surface Tension of Aqueous Solutions). For further Guidance see Volume I Part A of *Guidance on information requirements for biocides*. Further Guidance:

- *Guidance on information requirements and chemical safety assessment Chapter R7a: Endpoint specific guidance, R.7.1.6 Surface Tension.*

## **3.9. Viscosity**

These data are always required for liquid products; the relevant test method is OECD guideline 114 (Viscosity of Liquids).

Further guidance:

- *Guidance on information requirements and chemical safety assessment Chapter R7a: R.7.1.18 Viscosity,*

#### **4. Physical hazards and respective characteristics**

##### **4.1. Explosives**

Explosivity properties need to be determined, unless it can be justified that it is technically or scientifically not necessary to perform such studies.

An acceptable justification for non-performance of a test for explosive properties is where none of the components have explosive properties, and where available thermodynamic information establishes beyond reasonable doubt that the product is incapable of exothermic reaction.

##### **4.2. Flammable gases**

Not relevant for products containing micro-organisms.

##### **4.3. Flammable aerosols**

Flammability of aerosols dispensers must be determined by the classification criteria and test methods described in the section 2.3 of Annex I to the CLP Regulation.

An acceptable justification for non-performance of a test for flammability properties is where none of the components are classified as flammable.

##### **4.4. Oxidising gases**

Not relevant for products containing micro-organisms.

##### **4.5. Gases under pressure**

Not relevant for products containing micro-organisms.

##### **4.6. Flammable liquids**

Flash point and flammability must be determined, unless it can be justified that it is technically or scientifically not necessary to perform such studies.

An acceptable justification for non-performance of a test for flammability properties is where none of the components are classified as flammable.

##### **4.7. Flammable solids**

Flash point and flammability must be determined, unless it can be justified that it is technically or scientifically not necessary to perform such studies.

An acceptable justification for non-performance of a test for flammability properties is where none of the components are classified as flammable.

##### **4.8. Oxidising liquids**

Oxidising properties need to be determined, unless it can be justified that it is technically or scientifically not necessary to perform such studies.

##### **4.9. Oxidising solids**

Oxidising properties need to be determined, unless it can be justified that it is technically or scientifically not necessary to perform such studies.

##### **4.10. Organic peroxides**

Not relevant for products containing micro-organisms.

##### **4.11. Corrosive to metals**

Corrosivity towards metals may become relevant when anaerobic, hydrogen-consuming micro-organisms are employed as biocidal agents.

#### **4.12. Other physical indications of hazard**

##### **4.12.1. Auto-ignition temperatures of products (liquids and gases)**

Auto-ignition temperature is relevant for all liquid products.

##### **4.12.2. Relative self-ignition temperature for solids**

Relative self-ignition temperature is relevant for all solid products.

##### **4.12.3. Dust explosion hazard**

A dust explosion hazard is applicable to all powders and products containing, or able to produce dust that can either ignite or explode when exposed to an ignition source when dispersed in air (relevant for particulates up to 1 mm in diameter).

### **5. Methods of detection and identification**

#### **Introduction**

This section covers analytical methods required for post-registration control and monitoring purposes from the use of the biocidal product. For the analysis of the biocidal product the requirements cover the determination of the micro-organism and chemical components of the biocidal product on storage and a method required for post registration control and monitoring.

Information on analytical methods is required for assessing compliance with conditions for issuing authorisation for a biocidal product according to Article 19(1)(c) of the BPR. For products which are difficult to analyse, a description of the problems should be given.

Both production and product must be subject to a continuous quality control by the manufacturer. The quality criteria for the product should be submitted. For analytical methods used for generation of data as required in the BPR or for other purposes, for instance monitoring purposes, the applicant has to provide a justification for the method used.

Descriptions of methods must be provided and include details of equipment, materials and conditions used.

#### **5.1. Analytical method for determining the concentration of the micro-organism(s) and substances of concern in the biocidal product**

Analytical methods for the determination of the micro-organism in the biocidal product and, where relevant, chemical compounds used for the biocidal product as well as contaminants possible present in the biocidal product must be submitted.

The micro-organism, as well as co-formulants, may not be detectable or less detectable when contained within or adhered to the formulation (instead of being the sole compound to be detected). The applicant should therefore take into consideration that methods for analysis of the biocidal product and methods to determine and quantify residues (endpoint 5.2 of Annex III Title 2 for biocidal product) may differ and should highlight relevant differences.

#### **5.2 In so far as not covered by Annex II 4.2, analytical methods for monitoring purposes (ADS)**

Annex III, Title 2, 5.2 Column 1 states: *Analytical methods for monitoring purposes including recovery rates and the limit of quantification and detection for the active substance and other products where relevant, in/on food of plant and animal origin or feeding stuffs and (not necessary if neither the active substance nor the article treated with it does not come into contact with food producing animals, food of plant and animal origin or feeding stuffs)*

Analytical methods for the determination of residues, as defined in Annex II, Title 2, endpoint 4.2 of the BPR, must be submitted unless it is justified that the information already submitted according to that requirement or to the requirements of Annex II, Title 2, endpoint 7.7 and Annex III, Title 2, endpoint 8.9 of the BPR, is sufficient.

## **11. Measures necessary to protect humans, animals and the environment**

### **11.1. Recommended methods and precautions concerning: handling, storage, transport or fire**

Please follow the guidance in relation to section 10 Annex II Title 2 for active micro-organism. Further consideration should be given to specific co-formulants of the final formulation which are not considered under section 10 Annex II Title 2 for active micro-organism yet they may crucially determine the biocidal product's properties with regard to fire, particularly suspending oils or carrier substances of powders.

#### **11.2. Measures in the case of an accident**

Please follow the guidance in relation to section 10 Annex II Title 2 for active micro-organism. Further consideration should be given to specific co-formulants of the final formulation which are not considered under section 10 Annex II Title 2 for active micro-organism, yet they may crucially determine the biocidal product's properties with regard to fire, particularly suspending oils or carrier substances of powders.

#### **11.3. Procedures for destruction or decontamination of the biocidal product and its packaging**

Please follow the guidance in relation to section 10 Annex II Title 2 for active micro-organism. Further consideration should be given to specific co-formulants of the final formulation which are not considered under section 10 Annex II Title 2 for active micro-organism, yet they may crucially determine the biocidal product's properties with regard to fire, particularly suspending oils or carrier substances of powders.

##### **11.3.1. Controlled incineration**

The title of this data point is chemically orientated, as for biocidal product containing micro-organisms direct incineration is not the preferred waste disposal method.

The suggested waste disposal method is decontamination of the biocidal product by autoclave sterilization, UV-radiation or immersion baths containing disinfectants prior to a disposal with the household waste which could be later on incinerated in a controlled manner by a licensed incinerator. Attention should be paid to products containing spore-forming micro-organisms. Inactivation of bacterial spores requires special methods. The properties of the biocidal product deriving from its co-formulants should also be taken into consideration.

If an incineration is preferred, recommendations for burning conditions (e.g. temperature, reaction time, oxygen content) and other information needed for the safe incineration of the waste must be given. In addition information on compounds generated by burning and their disposal must be provided.

##### **11.3.2. Others**

Other methods to dispose biocidal products, packaging and contaminated materials must be fully described. Data must be provided for such methods, to establish their effectiveness and safety.

#### **11.4. Packaging and compatibility of the biocidal product with proposed packaging materials**

Packaging to be used must be fully described and specified in terms of the materials used, manner of construction (e.g. extruded, welded etc.), size and capacity, size of



opening, type of closure and seals. It must be designed in accordance with the criteria and guidelines specified in the FAO "Guidelines for the Packaging of Pesticides".

The suitability of the packaging, including closures, in terms of its strength, leak proofness and resistance to normal transport and handling, must be determined and reported according to ADR Methods 3552, 3553, 3560, 3554, 3555, 3556, 3558, or appropriate ADR Methods for intermediate bulk containers, and, where for the biocidal product child-resistant closures are required, according to ISO standard 8317.

#### **11.5. Procedures for cleaning application equipment where relevant**

The procedures should be such that the likelihood of accidental contamination of water or its sediments is minimised.

Cleaning procedures for both application equipment and protective clothing must be described in detail. The effectiveness of the cleaning procedure must be determined, using for example, biotests, and reported.

#### **11.6. Monitoring plan to be used for the active micro-organism and other micro-organism(s) contained in the biocidal product including handling, storage, transport and use**

Please follow the guidance for section 4.2 Annex II Title 2 of the BPR for active micro-organisms.

### **12. Classification, labelling and packaging. Example labels, instructions for use and safety data sheets shall be provided**

If the product contains a GMO (Genetically Modified Organism) or GMOs the product has to be marked with the sentence "The biocidal product contains a genetically modified organism" or accompanying documents must be provided which point to this fact according to Article 13(2)(f) of the Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC.

The classification and labelling of chemical co-formulants has to be treated separately and follow the standard rules for chemicals.

Generally, all micro-organisms are considered to be potentially sensitizing and should be labelled with "Micro-organisms may have the potential to provoke sensitising reactions.". If scientific evidence shows that a microorganisms do not have sensitization potential, this warning phrase could be waived<sup>15</sup>. In case the product contains sensitisers, then classification (and appropriate hazard statement) may be needed instead of the warning phrase.

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<sup>15</sup> Discussed at the CTGB Workshop on the toxicological risk assessment of pesticides using microorganisms (November 2015).

### **12.1. Indication on the need for the biocidal product to carry the biohazard sign specified in Annex II to Directive 2000/54/EC**

Proposals for the allocation of the biocidal product to one of the risk groups outlined in Article 2 of Directive 2000/54/EC<sup>16</sup> of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC<sup>17</sup>) with justifications for the proposal must be provided. According to this classification the biocidal product has to be labelled and carry the biohazard sign.

### **12.2. Precautionary statements including prevention, response, storage and disposal**

According to Article 17 of the CLP Regulation, a mixture classified as hazardous must bear a label which includes relevant precautionary statements in accordance with Article 22, where applicable.

As outlined in Annex I and Annex IV, the CLP Regulation recognizes five types of precautionary statements; these are (1) general statements, (2) prevention statements, (3) response statements, (4) storage statements, and (5) disposal statements.

For further information on the precautionary statements please refer to the *Guidance on Labelling and Packaging in accordance with Regulation (EC) No 1272/2008*<sup>18</sup>.

Further guidance:

- EU Evaluation Manual for the authorisation of biocidal products v2.0 (available on ECHA Website)<sup>19</sup>

### **12.3. Proposals for safety data sheets should be provided, where appropriate**

Safety data sheets (SDSs) are a well-accepted and effective method for the provision of information to recipients of substances and mixtures in the EU. SDSs for TGAI and biocidal products should be prepared and made available in accordance with Article 31 of REACH, where applicable<sup>20</sup>.

The SDS follows a 16 section format which is internationally agreed. The SDS must be supplied in an official language of the Member State(s) where the substance or product or treated article is placed on the market, unless the Member State(s) concerned provide(s) otherwise.

The SDS needs to be supplied for microbial active substance and biocidal products or treated article.

Further guidance:

- *Guidance on the compilation of safety data sheets*

### **12.4. Packaging (type, materials, size, etc.), compatibility of the product with proposed packaging materials to be included**

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<sup>16</sup> Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC)

<sup>17</sup> Council Directive 89/391/EEC of 12 June 1989 on the introduction of measures to encourage improvements in the safety and health of workers at work

<sup>18</sup> Guidance documents on CLP are available at <http://echa.europa.eu/web/guest/guidance-documents/guidance-on-clp>.

<sup>19</sup> <http://echa.europa.eu/web/guest/guidance-documents/guidance-on-biocides-legislation>

<sup>20</sup> The reader is reminded that microorganisms are not included within the scope of the definition of a substance under REACH and are therefore outside the scope of the REACH Regulation.

This endpoint is redundant as it is already addressed under endpoint 11.4.

### 13. Summary and evaluation

The key information identified from the endpoints in each subsection (2-12) is summarised, evaluated and a draft risk assessment is performed.

The summary and evaluation must be provided in separate assessment documents attached to the IUCLID file (the templates are available on the ECHA website).

The Decision on technical equivalence if relevant should be also provided.

## 3.2 Part B: Hazard and risk assessment

### 3.2.1 Active Micro-organism

#### 3.2.1.1 Identity of the active micro-organism

It is important to establish the identity of the active micro-organism in order to distinguish it from other micro-organisms, especially those that are pathogenic and in other ways potentially harmful. The ability to adequately establish the identity is necessary also, for example, for the assessment of residues remaining on treated surfaces or for monitoring purposes in the environment.

As taxonomy is currently changing, mainly due to the transition to DNA sequence analysis for use in systematics, the names of micro-organisms may change as well as the species affiliation. Taxonomy based on genotype may thus bring a change from what was originally proposed for the micro-organism in the application. Such change may include a change at species or strain level and this may have consequences when read-across of data has been used between strains assumed by the time to be closely related<sup>21</sup>.

Many micro-organisms have the potential to produce a wide range of secondary metabolites and some of these have harmful properties. The production of metabolites usually depends on the prevailing growth conditions and they can be induced for instance in the presence of the target organism, in the presence of certain nutrients, during starvation, at certain growth temperatures, pH, etc. Whether or not these metabolites remain in the TGAI depend on the technique employed during manufacturing process. The identity of the TGAI refers to the micro-organism produced by a specific production process and any relevant metabolites/toxins formed in the process as well as any fermentation residues and contaminants present. To clarify the distinction from the micro-organism found in nature, the term technical grade active ingredient (TGAI) is used.

In order to assess potential environmental and human health risks, information on the amount of TGAI, including viable and/or non-viable residues, resulting from the intended use is needed. This requires sensitive and reliable analytical methods. Since these may be specific to the micro-organism, the acceptability of methods needs to be based on expert judgement. However, the sensitivity of the method needs to be considered, for example, the method may need to be able to distinguish between the amount of residues present before application (i.e. the natural background level) and after. Sequencing of the full genome should not be always requested, but limited genetic information at species/strain level would be considered sufficient. However, considering that full genome sequencing nowadays is a fairly straightforward analysis made at a

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<sup>21</sup> This was the case for three *Trichoderma* strains evaluated under 91/414/EC. These strains were reclassified and regarded as phenotypically identical but genetically different (discussed at EFSA PRAPeR M05 2012).

reasonable cost, this should be encouraged to confirm the identity of the active micro-organism. This information can also be used to check if it contains genes encoding toxins that are known to be produced by related strains. While the presence of such genes does not necessarily mean that the gene will be expressed, an absence of genes could be used as evidence that the toxin in question would not be produced.

Any other known information, either on the strain or on the species, which identifies the TGAI must be submitted. Incorporating the production method as an identifier (if confidential business information (CBI) rules allow) may also aid in identification and enables changes in manufacturing site (if authorised) at a later stage using the same production method with respect to micro-organism identification.

The deposition of the strain at an internationally recognised culture collection must be checked.

From the information provided in section 4.1.1, a profile of the biological properties of the TGAI can be obtained. This profile is very important to be considered in the human health and environmental hazard and risk assessment. If, for example, in section 4.1.1 it is concluded that the micro-organism is unable to grow at temperatures comparable to human or animal body temperatures, then the infective potential in humans and animals can be expected to be low. Furthermore, if data indicate that the micro-organism is likely to produce toxic metabolites, the additional data requirements in the human health section may apply. Information in section 4 may also indicate that the intentional application could be expected to cause a disturbance of the biological balance in the environment. In that case factors such as persistence during prevailing conditions at the application site may need further consideration.

### 3.2.1.2 Biological properties of the active micro-organism

#### 3.2.1.2.1 Origin, natural occurrence and life cycle of the active micro-organism

The origin of the strain, and where relevant, its natural habitat including indications on the natural background level, life cycle and the possibilities for survival, colonisation, reproduction and dispersal must be evaluated. Proliferation of an indigenous micro-organism applied for biocontrol is expected to cease and return to normal background levels after an initial short growth period.

The ability of active micro-organisms to adapt to the environment must be assessed. In particular the following principles should be considered:

- a) depending on the prevailing conditions at the site of application (e.g. availability of substrates for growth and metabolism) micro-organisms can switch on or off the expression of different genes. In combination with other environmental factors, this may result in an altered phenotype;
- b) the microbial strains most adapted to the environment can survive and multiply better than the non-adapted strains. Adapted strains have a selective advantage and can form the majority within a population after a number of generations;
- c) the relatively rapid multiplication of micro-organisms leads to a higher frequency of mutations. If a mutation is promoting survival in the environment, the mutant strain can become dominant;
- d) the properties of viruses, in particular, can change rapidly, including their virulence.

Therefore, where appropriate, information on the genetic stability of the active micro-organism under the environmental conditions of proposed use must be assessed, as well as information on the active micro-organism's capacity to transfer genetic material (e.g. plasmids or elements that confer antibiotic or disinfectant resistance) to other organisms

and information on the stability of encoded traits. Where there is reason for concern, all available information on the potential for transfer of genetic material to other species (primary concern usually plasmids between bacteria species) should be provided to identify level of risk.

#### **3.2.1.2.2 Mode of action and metabolite/toxin production.**

The mode of action of the active micro-organism should be assessed in as much detail as appropriate for the risk assessment. The possible role of relevant metabolites/toxins for the mode of action should be assessed and when identified, information on the minimal effective concentration for each active metabolite/toxin could be considered if available. Information on mode of action can be a very valuable tool in identifying potential risks and would also help design relevant risk assessment. Aspects to be considered in the assessment are:

- a) antibiosis;
- b) induction of tolerance, resistance/cross-resistance;
- c) interference with the virulence of a pathogenic target organism;
- d) colonisation ability;
- e) competition of ecological niche (e.g. nutrients, habitats);
- f) parasitism; and
- g) vertebrate and invertebrate pathogenicity.

#### **3.2.1.2.3 4 Growth temperature**

The growth temperature is of great importance to human health risk. If the growth temperature requirement of the micro-organism is comparable to human body temperature, this may indicate a potential for infectivity. In contrast, a growth temperature requirement incompatible with human body temperature could indicate a low concern for infectivity. Growth temperature could also support a low concern of infectivity of homeothermic animals (other than human). Therefore, the minimum and maximum growth temperatures should be established as well as viability of the organism at 37°C. The growth temperature has been considered to be a strain specific parameter that cannot be extrapolated between strains. Just knowing the growth temperature is not enough for waiving toxicological studies but could be used to read across infectivity data. Phenotype similarity together with growth temperature can be enough to extrapolate infectivity data from one strain to another<sup>22</sup>.

#### **3.2.1.2.4 Host specificity range and pathogenicity potential**

In order to assess possible effects on non-target organisms, information on the active micro-organism's host specificity must be assessed, taking into account the characteristics and properties described in (a) and (b):

- a) The ability of an active micro-organism to be pathogenic for non-target organisms (humans, animals, and other non-target organisms) must be assessed. Any relationship to known plant, animal or human pathogens that are species of the genus of the active and/or contaminating micro-organisms must be assessed. A characteristic gene sequence or a few characteristic gene sequences for a microbial species or strain should be sufficient for example, to prove/disprove relationship of a micro-organism to a known pathogen.

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<sup>22</sup> Discussed at EFSA PRAPeR M05 2012.

- b) Pathogenicity as well as virulence is strongly related to the host-species (e.g. determined by body temperature, physiological environment) and to the host conditions (e.g. health condition, immune status). For example, multiplication in humans depends upon the ability of the active micro-organism to grow at the body temperature of the host. A psychrophilic micro-organism is unlikely to survive or replicate at human body temperatures. However, the route of entry of the active micro-organism into the host (oral, inhalation, skin/wound) can also be the critical factor. For example, a microbial species may cause a disease following entry via skin damage, but not via the oral route.

Many micro-organisms produce antimicrobial substances that cause normal interferences in the microbial community. Resistance to antimicrobial agents of importance for human and veterinary medicine must be assessed, and the possibility for transfer of genes that code for resistance to these antimicrobial agents should be assessed.

### **3.2.1.3 Quality control of the production of the micro-organism in the biocidal product**

The quality assurance criteria proposed for production of the active micro-organism must be assessed. In the assessment criteria relating to process control, good manufacturing practice, operational practices, process flows, cleaning practices, microbial monitoring and hygiene conditions should be taken into account to ensure good quality of the active micro-organism. The quality, stability, purity, etc., of the active micro-organism must be addressed in the quality control system.

Full information need to be provided on the continuous quality control of the production method, production process and biocidal product. In particular, the occurrence of spontaneous changes in major characteristics of the active micro-organism and the absence/presence of contaminating organisms must be considered. The quality assurance criteria for production and the techniques used to ensure a uniform biocidal product must to the extent possible be described and specified.

The quality control process should reassure that primarily the inoculum as well as the culture should be free of contaminants or contain minor contaminant levels; some guidance about the contaminant limit criteria is available in: OECD Series on Pesticides No 65 issue paper, however the criteria and the suitability of methods should be judged on a case by case basis. The proposed OECD microbial contamination screening requirements, which employ standard methods from the food industry, provide practical microbiological specifications for microbial pest control products, and provide meaningful data to evaluate the overall acceptability of microbial pest control products without posing an unreasonable burden on applicants/notifiers. Authorities can be reasonably assured that products which meet these microbiological specifications do not contain microbial contaminants which will pose a risk to human health or to the environment.

## **3.2.2 Biocidal Product**

### **3.2.2.1 Identity of the biocidal product**

The detailed quantitative and qualitative information provided on the composition of the biocidal product, such as that concerning the micro-organism, relevant metabolites/toxins, residual growth medium, co-formulants and microbial contaminants present should be taken into account in the hazard assessment.

### **3.2.2.2 Physical, chemical and technical properties of the biocidal product**

Depending on the nature of the active micro-organism and the formulation type, the technical properties of the biocidal product must be assessed. For example, for a powder formulation information on dustability needs to be submitted.

The formulation type is important for exposure assessment. The human and environmental exposure to formulations containing the same amount of the active substance would be expected to differ substantially if the active substance is encapsulated and slowly released or included in a soluble concentrate applied as a spray.

Shelf-life and storage stability of the preparation should be assessed, taking into account possible changes in composition such as growth of the active micro-organism or of contaminating micro-organisms, production of relevant metabolites/toxins, etc.

Where the proposed label claims include requirements or recommendations for use of the preparation with other biocidal products or adjuvants as a mixture, and/or where the proposed label includes indications concerning the compatibility of the preparation with other biocidal products as a mixture, those biocidal products or adjuvants must be physically and chemically compatible in the mixture. Biological compatibility must also be demonstrated for the mixtures, i.e. it must be shown that each biocidal product in the mixture performs as expected and that no antagonism occurs.

### **3.2.2.3 Quality control of the biocidal product**

The quality assurance criteria proposed must be assessed. Detailed information on the production process must be given.

## **3.3 Part C: Evaluation/conclusion/decision criteria**

### **3.3.1 Active Micro-organism**

#### **3.3.1.1 Evaluation of data on identity, biological and technical properties**

For each authorisation granted the Member States must ensure that the micro-organism concerned is deposited at an internationally recognised culture collection and has an accession number. The micro-organism must be identified and named at the strain level. Identification and characterisation should be done at strain level but data on the species may be acceptable if justified. Examples for such justifications can be genera of micro-organisms of closely related species, like the genus *Bacillus*. In such cases it can be difficult to differentiate single strains. Further examples are represented by genera of micro-organisms which do not include pathogenic species or species relevant for human health or environment. In addition there might be genera of micro-organisms for which hardly any experimental method for differentiation of strains exists.

There must also be information as to whether or not the micro-organism is a wild type or a spontaneous or induced mutant, or a genetically modified organism.

The mode of action of the active micro-organism should be assessed in as much detail as appropriate for the risk assessment.

#### **3.3.1.2 Evaluation of analytical methods**

Member States must evaluate the methods proposed to identify the specific strain concerned and especially methods that discriminate that strain from closely related strains.

For drinking water quality: Information on lack of interference (e.g. that may result in false negative or positive results) of micro-organism on methods of analysis for pathogens in drinking water should be routinely provided.

#### **3.3.1.3 Decision criteria**

No authorisation must be granted if on the basis of the information provided in the dossier it appears that the micro-organism is pathogenic to humans and/or poses

unacceptable risks to non-target organisms. However, in case a risk is identified for particularly sensitive target groups only (e.g. immunocompromised people, elderly), a decision should be based on case-specific considerations taking into account, for example, case reports for the species, test results, likelihood of exposure and other relevant information.

### **3.3.2 Biocidal Product**

#### **3.3.2.1 Evaluation of data on identity, biological and technical properties**

There must be sufficient information to permit evaluation of the minimum content of the active micro-organism in the material used for the manufacturing of biocidal products, as well as in the biocidal product. Information of maximum content should be reported if concern for human health or environment exists due to exposure to the microorganism, or if secondary toxins/metabolites are produced. The content of co-formulants and relevant metabolites/toxins in the biocidal product and contaminating micro-organisms derived from the production process must be defined. Member States must ensure that the level of contaminating organisms is controlled to an acceptable level. In addition: the physical nature and state of the biocidal product must be specified.

#### **3.3.2.2 Evaluation of analytical methods**

Member States must evaluate the analytical methods proposed to identify and quantify the toxicologically, ecotoxicologically or environmentally relevant viable and non-viable components resulting from the micro-organism and/or present as impurity or co-formulant (including eventually resulting breakdown and/or reaction products thereof).

The methods proposed for identification/detection and quantification must reflect the most appropriate justified technology. Methods for post-authorisation monitoring should involve the use of commonly available reagents and equipment.

An adequate method of sufficient quality to identify and quantify the micro-organism and non-viable components (e.g. relevant metabolites, impurities and co-formulants) in the biocidal product down to below known or anticipated levels of concern is necessary. In the case of a biocidal product containing more than one micro-organism as active ingredient, the recommended methods should be capable of identifying and determining the content of each one.

Adequate methods for authorisation control and monitoring of viable and/or non-viable residues are necessary. Authorisation control must be based on an adequate monitoring plan. Methods must be available for analysis of:

- a) material, foodstuff and feedstuffs if toxicologically relevant residues occur. Residues are considered relevant if a maximum residue level (MRL) or a waiting<sup>23</sup> or re-entry safety period or other such precaution is required;
- b) soil, water, air and/or body tissues if toxicologically, ecotoxicologically or environmentally relevant residues occur.

#### **3.3.2.3 Decision criteria**

No authorisation will be granted if on the basis of the information provided in the dossier it appears that the product has unacceptable effects on humans or on non-target

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<sup>23</sup> The occurrence of a waiting period should not automatically trigger the need for residue testing. Liquid formulations often have a waiting period included in use recommendations to allow the product to dry after application; this therefore relates to product performance rather than safety issues.



organisms under the proposed conditions of use. In case risk is identified for vulnerable groups among the different populations, a decision should be taken on a case-by-case basis (see section 3.3.1.3).

## 4. Effectiveness against target organism

### 4.1 Part A: Information requirements

#### 4.1.1 Active Micro-organism

**NOTE to the reader:** The endpoints described below are numbered in accordance with the BPR, Annex II Information Requirements for Active Substances, Title 2 Micro-organisms, Core data set and additional data set for active substances.

Headings are shown in *italic green font* to distinguish them from the general section numbers of the Guidance document.

#### *5. Effectiveness against target organism*

##### *5.1. Function and mode of control e.g. attracting, killing, inhibiting*

The information provided must describe the intended purposes for which biocidal products containing the active micro-organism is to be used.

##### *5.2. Infectiveness, dispersal and colonisation ability*

Influence of typical conditions of use on survival, growth, colonisation, and effectiveness of the active micro-organism must be stated.

##### *5.3 Representative organism(s) controlled and products, organisms or objects to be protected*

For an organism to be controlled, provide both the common name and the scientific name when possible and also the sex, strain and stadia where relevant and appropriate. Where complexes of organisms are involved, generic names, representative of the diversity of the complex must be indicated. Where human and/or animal pathogens are involved, the specific name(s) must be provided.

Indicate in which parts of EU the organisms to be controlled exist.

##### *5.4 Effects on representative target organism(s) Effects on materials, substances and products*

###### *Effects on representative target organism(s)*

The effects on the target organisms required for the claimed efficacy should be described and specified if possible for each use and method of application if these have different effects.

The dependence of the effect on the concentration of the active micro-organism, host specificity and life cycle in the relevant environment should be indicated.

###### *Effects on materials, substances and products*

If there is evidence of unintended effects on non-target materials, substances or products, information must be submitted.

##### *5.5 Likely concentration at which the micro-organism will be used*

The likely use concentration in the target area should be stated for each use and method of application. Indicate if the use concentration should be different in different parts of EU. The likely concentration at which the active micro-organism will be used must, where appropriate, be accompanied by the likely concentration of the relevant biocidal substances produced by the micro-organism.

Justification for the selection of the use concentration should be provided. The likely use concentration should ideally be the minimum effective density, taking into account all

relevant parameters that impact on efficacy.

### **5.6 Mode of action (including time delay)**

The principal mode of action should be indicated in as much detail as technically feasible. It should be indicated if the mode of action is by invasion of the target host including parasitism or by competition, repellency, or degradation by enzymes in the target organism. In connection with the mode of action it should also be stated if the micro-organism produces a toxin with a residual effect on the (parasitism) target organism. In the case of a relevant metabolite/toxin clearly involved in the mode of action, this relevant metabolite/toxin should be described. If appropriate, information on the site of infection and mode of entry into the target organism and its susceptible stages should be given. The results of any experimental studies must be reported. Information on environmental conditions such as temperature, pH and other factors that could affect the mode of action, and particularly the production of toxic metabolites, should also be reported.

If relevant, the way of uptake of the micro-organism, or its relevant metabolites (in particular toxins) (e.g. contact, stomach, inhalation) should be indicated. It must also be stated whether or not the micro-organism or its relevant metabolites/toxins are translocated in other organisms and, where relevant, how this translocation takes place.

### **5.7. Efficacy data**

The efficacy of the active micro-organism is determined by the level of control it exerts on the target organism. A high infectivity rate and a high level of mortality of the target organism will increase the efficacy of the active micro-organism. The active micro-organism could also exert a controlling effect by competition with the other target organisms or by other biochemical means control the activity of the target organism.

To assess the efficacy of the TGAI, the general guidance for chemical biocides<sup>24</sup> can be consulted. Efficacy can be demonstrated by data obtained from laboratory studies, pilot plants, field test data or other relevant study data, the test conditions of which are comparable with the purpose applied for and which are comparable with the environmental characteristics relevant for the intended use. The test method should measure a response and, as appropriate, an end-point relevant to the claims made. The method should employ an untreated control and the efficacy test reports should contain dose response data for dose rates lower than the recommended rate. However, this may not be always possible for field studies.

For field studies conducted outside the territory of the Member State in which the authorisation is being sought, a justification of the relevance of such data must be made. The extent of the information required will vary depending on the product type and proposed use pattern and upon the similarity of the conditions in the two countries. Justification may include, as relevant and appropriate, information on the target organism (e.g. comparison of genera/species and its relevance to the Member State in which authorisation is sought), meteorological parameters (e.g. mean temperatures and rainfall) and location details.

### **5.8. Any known limitations on efficacy**

For this endpoint the general guidance for biocidal products could be consulted. This guidance recommends that possible factors that can reduce the efficacy, for instance hot, cold or humid environments or the presence of other substances should be stated along with the reason for these. Information on the occurrence or possible occurrence of

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<sup>24</sup> *Guidance on the Biocidal Products Regulation Volume II: Efficacy Part A: Information Requirements.*  
Available at: <http://echa.europa.eu/web/guest/guidance-documents/guidance-on-biocides-legislation>.

development of resistance, including cross-resistance, and appropriate management strategies should be stated. This information must be submitted even where it is not directly relevant to the uses for which authorisation is sought or to be renewed (e.g. different species of harmful organism), as it may provide an indication of the likelihood of resistance development in the target population. Furthermore, the guidance recommends that observations of undesirable or unintended side effects for example, on beneficial and other non-target organisms and adverse reaction to materials should be provided as far as this is not covered elsewhere. Where information on unnecessary suffering and pain for target vertebrates has been observed, it should be reported although no additional testing should be conducted specifically to assess the suffering and pain of vertebrates.

#### ***5.8.1. Information on the occurrence or possible occurrence of the development of resistance of the target organism(s) and appropriate management strategies***

Available information on the possible occurrence of the development of resistance or cross-resistance of the target organism(s) must be provided. Where possible, appropriate management strategies should be described.

#### ***5.8.2. Observations on undesirable or unintended side effects***

Provide observations on undesirable or unintended side effects. Provide observations such as unnecessary suffering and pain for vertebrates where such information is available. It should also be reported if the active micro-organism is anticipated to have adverse effects on animate or inanimate substrates or matrices not directly related to the intended use. Spreading of an infection to non-target organisms can be seen as an unintended side effect.

#### ***5.8.3. Host specificity, range and effects on species other than the target organism***

Information on the infectivity and pathogenicity of the active micro-organism to the target organism(s) and other non-target organism(s) is required. More details on the host interaction should be presented under the section dealing with mode of action.

In case of pathogenic effect on the target organism, infective dose (the dose needed to cause infection with the intended effect on a target species) and transmissibility (possibility of spread of the active micro-organism in the target population, but also from one target species to another (target) species) after application under the proposed conditions of use must be indicated.

Available information on the effects on non-target organisms being either closely related to the target species or being especially exposed within the area to which the active micro-organism(s) may spread must be given.

To be noted that studies testing the effects on non-target organisms are not always relevant and therefore not always required. The need for non-target testing, should be considered on a case by case basis. When no effect on non-target organism is expected, absence of testing can be justified.

#### ***5.9. Methods to prevent loss of virulence of seed stock of the micro-organism***

Methods to prevent loss of virulence of starting cultures are to be provided. In addition, any method, if available, that could prevent the active micro-organism from losing its effects on the target species must be described. Fast and reliable methods should be available to prove that virulence of seed stock (cells, spores) of the micro-organism is still present.

## **6. Intended uses and exposure**

### **6.1. Field of use(s) envisaged**

Use means all operations carried out with a biocidal product, including storage, handling, mixing and application. Uses taking place outside the Union should be disregarded. Any operation carried out with a view to exporting the biocidal product or the treated article outside the Union should also be disregarded.

The intended and possibly potential use should be indicated together with the fields of use. In addition, give a detailed description of the overall use patterns. Information on the fields of use envisaged should be sufficient to allow for an approximate but realistic estimation of human and environmental exposure to the active micro-organism.

### **6.2. Product-type(s)**

The intended and potential product-type(s) as listed in BPR Annex V should be indicated.

### **6.3. Detailed description of the intended use pattern(s)**

Provide a detailed description of the overall use pattern(s) linked to the fields of use envisaged. This information should be sufficient to allow for an approximate but realistic estimation of human and environmental exposure to the active substance under realistic worst case conditions.

### **6.4. Category of users for which the micro-organism should be approved**

Indicate users with the help of the following user categories:

- Industrial user: user involved in manufacturing, handling and/or packaging of actives or products at industrial sites;
- Trained professional: professional user using end-products outside industry with a licence<sup>25</sup>;
- Professional user: professional user using end-products outside industry;
- Non-professional user: member of the general public at a workplace or at home (consumer).

Users outside the Union should be disregarded.

The following is an example of the use/user category:

- mosquito larvae control, used against public health pests in forests or parks, and in animal housing/professional user.

### **6.5. Exposure data applying, as appropriate, the methodologies described in section 5 of Annex I to Regulation (EC) No 1907/2006**

#### **6.5.1. Information on human exposure associated with the intended uses and disposal of the active substance**

The provided information should be sufficient to allow an approximate but realistic estimation of human (occupational and consumer) exposure associated with the proposed/expected uses and disposal of an active substance. The prediction of the exposure levels should also describe a reasonable worst case situation, excluding accidental exposure and abuse.

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<sup>25</sup> To be noted that in many countries a recognised certification for users of biocides does not exist yet. Therefore a professional may have received valid training but no certification.


### **6.5.2. Information on environmental exposure associated with the intended uses and disposal of the active substance**

The provided information should be sufficient to allow an approximate but realistic estimation of environmental exposure associated with the proposed/expected uses and disposal of an active substance. The prediction of the exposure levels should also describe a reasonable worst case situation, excluding accidental exposure and abuse.

### **6.5.3. Information on exposure of food producing animals and food and feeding stuffs associated with the intended uses of the active substance**

There is currently no guidance available, neither a specific guidance for micro-organisms nor a general guidance for biocides. However, guidance documents being developed within the BPC Ad hoc Working Group on the Assessment of Residue Transfer to Food<sup>26</sup> related to chemical substances may be consulted if considered relevant.

## **4.1.2 Biocidal Product**

 **NOTE to the reader:** The endpoints described below are numbered in accordance with the BPR, Annex III Information Requirements for Biocidal Products, Title 2 Micro-organisms, Core data set and additional data set for active substances.

Headings are shown in *italic green font* to distinguish them from the general section numbers of the Guidance document.

## **6. Effectiveness against target organism**

### **6.1. Function and mode of control**

The information provided must describe the intended purposes for which biocidal product containing the active micro-organism is to be used.

### **6.2. Representative organism(s) to be controlled and products, organisms or objects to be protected**

Please follow guidance as specified in section 5.1.1 for the active micro-organism.

### **6.3. Effects on representative target organisms**

Information on the effects on the target organism(s) should be described and specified. In case different effects are expected due to different uses and different methods of applications, this should, if possible, be made for each use and method of application stated. Product-specific data should be presented if available but data on the active micro-organism or TGAI could be used provided it can be justified that data for the micro-organism/TGAI can be extrapolated to the product. The justification should take into account the intended doses and the composition of the product.

The dependence of the effect on the concentration of the active micro-organism, host specificity and life cycle in the relevant environment should be indicated.

### **6.4. Likely concentration at which micro-organism will be used**

The likely use concentrations in the target area should be stated for each use and method of application. Indicate if the use concentrations should be different in different parts of EU.

Justification for the selection of the use concentrations should be provided. The likely use concentration should ideally be the minimum effective concentration under real

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<sup>26</sup><http://echa.europa.eu/about-us/who-we-are/biocidal-products-committee/working-groups/assessment-of-residue-transfer-to-food>.

conditions for the respective service life, taking into account all relevant parameters that impact on efficacy.

### **6.5. Mode of action**

In case the mode of action is known or expected to differ in the presence of co-formulants, this should be reported and possible consequences should be discussed. Where available, the results of experimental studies must be reported.

### **6.6. The proposed label claims for the product**

The term "label claims" should include all claims made for the efficacy of the product, such as those on advertising material or accompanying leaflets, as well as those on the product label. A detailed evaluation of the efficacy data against the label claims should be carried out. The evaluation should include all relevant target species (or representative species), the effects of product usage, the duration and speed of effect, any claims for residual action, together with any other specific claims.

It should be noted that, in relation to the label claims, there are several pieces of information which will form part of the conditions of authorisation, such as:

- the formulation type (e.g. a solvent based ready-for-use, a water based concentrate, a dusting powder, a gel bait, etc.);
- application method, including equipment and/or dilution if appropriate (e.g. coarse spray, ultra-low volume (ULV) spray, skin lotion, etc.);
- application rate (e.g. apply 100 ml of product per square metre, spray for 20 seconds, etc.);
- frequency of treatment and any specific interval between applications;
- other specific conditions to be taken into account, e.g. occasionally, the "normal use" of a product may involve the use of the product in conjunction with other activities. This may include the cleaning of an area prior to treatment. The efficacy of products based on active micro-organism may depend on the environmental conditions. The conditions under which the product can best be used or should be used (e.g. temperature, RH, sunlight, time of day, time of year) should be indicated; any extra measurements which may be taken to improve the efficacy (e.g. keep wet, keep cool, add molasses etc.) should also be indicated. The contributions made by other components of an Integrated Pest Management procedure may also have to be taken into account.

### **6.7. Efficacy data to support these claims, including any available standard protocols, laboratory tests, or field trials used including performance standards, where appropriate and relevant**

The applicant must demonstrate that the biocidal product is effective and suitable for its intended use when applied according to its instructions for use. This can be confirmed by provision of data that may include laboratory studies, pilot plants, field test data or other relevant study data. The test conditions should be comparable with the purpose applied for and which are comparable with the environmental characteristics relevant for the intended use.

For field studies conducted outside the territory of the Member State in which the authorisation is being sought, a justification of the relevance of such data must be made. The extent of the information required will vary depending on the product type and proposed use pattern and upon the similarity of the conditions in the two countries. Justification may include, as relevant and appropriate, information on the target organism (e.g. comparison of genera/species and its relevance to the Member State in which

authorisation is sought), meteorological parameters (e.g. mean temperatures and rainfall) and location details.

The test method should measure a response and, as appropriate, an end-point relevant to the label claims. The method should employ an untreated control and, if possible, a reference product for comparison. The efficacy test reports should contain dose response data for dose rates lower than the recommended rate. However, this may not be always possible for field studies.

Where earlier formulations of the product or other products containing the same active micro-organisms are cited as supporting evidence, all relevant formulation details must be given and the relevance of this evidence to the current formulation must be fully justified.

### **6.8. Any other known limitations on efficacy including resistance**

Possible restrictions or recommendations concerning the use of the product in specific environmental or other conditions should be stated.

State possible factors that can reduce the efficacy of the product, such as hot, cold or humid environments or the presence of other substances. These factors should be accompanied by a robust explanation detailing why they negatively affect the product.

Possible recommendations concerning the avoidance of the continuous use of the product in order to prevent the development of resistant strains and the grounds for these should be stated.

State if the product cannot be mixed with, for example, other biocidal products or if the use of the product with other biocidal products is recommended.

#### **6.8.1. Information on the occurrence or possible occurrence of the development of resistance and appropriate management strategies**

Please follow guidance as specified in section 5.1.1 of this guidance document for the active micro-organism.

#### **6.8.2. Observations on undesirable or unintended side effects**

Please follow guidance as specified in section 5.1.1 of this guidance document for the active micro-organism.

## **7. Intended uses and exposure**

### **7.1. Field of use envisaged**

Please follow guidance as specified in section 5.1.1 of this guidance document for the active micro-organism.

### **7.2. Product-type**

Please follow guidance as specified in section 5.1.1 of this guidance document for the active micro-organism.

### **7.3. Detailed description of intended use**

Please follow guidance as specified in section 5.1.1 of this guidance document for the active micro-organism. The information provided here should be in agreement with the label claim (section 5.1.2, endpoint 6.6 for biocidal product).

### **7.4. User e.g. industrial, trained professional, professional or general public (non-professional)**

Please follow guidance as specified under endpoint 6.4 (section 5.1.1 of this guidance document) for the active micro-organism.



### **7.5. Method of application and a description of this method**

The method of application of the product for different uses, including the application equipment where appropriate, should be explained. If the product is to be diluted, the substance used for dilution and its volume, must be stated. A description of the application technique (e.g. dipping, spreading, spraying, automatic/manual dosing etc.) should be included. The substances that may have to be added to the solution and their dosages must also be given.

If certain technical device will be used together with product, a description of this device should be provided.

### **7.6. Application rate and if appropriate the final concentration of the biocidal product or the micro-organism active substance in a treated article or the system in which the product is to be used (e.g. in the application device or bait)**

The recommended dose of the product and the active micro-organism per object should be stated (e.g. per surface area of the material to be protected or as a concentration in a water system).

### **7.7. Number and timing of applications and duration of protection**

Annex III, Title 2, 7.7 Column 1 states: *Any particular information relating to the geographical location or climatic variations including necessary waiting periods for re-entry or necessary withdrawal period or other precautions to protect human and animal health and the environment.*

Indicate the number of applications, the frequency of treatment and the duration of protection.

Where relevant, it should be indicated how the applications differ in various areas of the EU due to the environmental differences. If such variations exist, then the recommended duration of application and possible re-applications for every area of use should also be indicated.

Further guidance:

- can be found in *Guidance on information requirements for biocides*.

### **7.8. Proposed instructions for use**

The proposed instructions for use of the biocidal product, to be printed on labels and leaflets, must be provided. The information provided here should be in agreement with the label claim (section 5.1.2 of this guidance document, endpoint 6.6 for biocidal product).

### **7.9. Exposure data**

Please follow guidance as specified under endpoint 6.5 (section 5.1.1 of this guidance document) for the active micro-organism.

#### **7.9.1. Information on human exposure associated with the proposed/expected uses and disposal**

Please follow guidance as specified under endpoint 6.5.1 (section 5.1.1 of this guidance document) for the active micro-organism.

#### **7.9.2. Information on environmental exposure associated with the proposed/expected uses and disposal**

Please follow guidance as specified under endpoint 6.5.2 (section 5.1.1 of this guidance document) for the active micro-organism.

## 4.2 Part B: Assessment

### 4.2.1 Active Micro-organism

#### 4.2.1.1 Assessment of the efficacy data

Data submitted by the applicant must be sufficient to substantiate the efficacy claims for the product. Data submitted by the applicant or held by the evaluating body must be able to demonstrate the efficacy of the biocidal product against the target organism when used normally in accordance with the conditions of authorisation.

For inclusion in the Union list of active substances approved for use in biocidal products in general only basic efficacy of the active substance is required (36<sup>th</sup> CA meeting March 2010<sup>27</sup>).

Testing should be carried out according to Union guidelines where these are available and applicable.

The TNsG on product evaluation (TNsG on Product Evaluation; Chapter 7) provides information per PT on assessment of chemicals which may be useful for active-micro-organisms too. The information in the TNsG will be incorporated into the development of the Biocides Guidance, Volume II Efficacy, Part B Assessment (for information on the status of biocides guidance development see the ECHA Biocides Guidance webpage<sup>28</sup>). The guidance specifies in which cases laboratory and field trials are needed to do the assessment and in which cases only laboratory or semi field trials are sufficient.

EPPO published a document on principles of efficacy evaluation for microbial plant protection products (OEPP/EPPO Bulletin (2012) 42 (3), 348–352). Also the OECD has published a document on the evaluation of microbials for pest control, which includes a chapter on efficacy (ENV/JM/MONO(2008)36). Although these documents are meant for plant protection products there may be useful information for biocidal active micro-organisms as well. Guidelines developed within the WHO may also be consulted.

It needs to be reminded that efficacy data retrieved from scientific literature should be relied upon with caution and considered as supportive information. These cannot replace data obtained from efficacy tests carried out according to standards.

#### 4.2.1.2 Assessment of the potential resistance to the active micro-organism

Information submitted by the applicant must be sufficient to assess the risk of development of resistance to the active micro-organism. Data for example, could include field studies, long-term studies, empirical values or literature values. Furthermore cross-resistance should be taken into consideration.

### 4.2.2 Biocidal Product

#### 4.2.2.1 Assessment of the efficacy data

The efficacy data provided to fulfil the requirements of Annex III Title 2 on the biocidal product of the BPR should be assessed having regard to the degree of control or the

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<sup>27</sup> The Role Of Efficacy In The Evaluation Of Active Substances For Annex I Inclusion. (Endorsed at the 36th meeting of representatives of Members States Competent Authorities for the implementation of Directive 98/8/EC concerning the placing of biocidal products on the market (10-12 March 2010). <http://echa.europa.eu/guidance-documents/guidance-on-biocides-legislation/biocidal-products-directive> (select "12. Additional guidance on specific issues").

<sup>28</sup> <http://echa.europa.eu/web/guest/guidance-documents/guidance-on-biocides-legislation>.

extent of the desired effect and having regard to relevant experimental conditions such as:

- a) the type of target organism the product is intended to control or any material known to be affected and material intended to be protected;
- b) the area to be protected;
- c) the environmental (including climatic) conditions existing in the area of use (if necessary for acceptable efficacy such data/information should also be given for the time before and after application);
- d) the presence and density of the harmful organism;
- e) the development stage of the organism and target organism;
- f) the amount of the microbial biocidal product used;
- g) if required on the label, the amount of adjuvant added;
- h) dilutant(s) and dilution rate if applicable;
- i) the frequency and timing of the applications;
- j) the type of application equipment;
- k) the need for any special cleaning measures for the application equipment.

The efficacy of the biocidal product needs to be assessed under the range of conditions – including climatic – and breeding period of the target organism likely to be encountered in practice in the area of proposed use. In particular, consideration should be paid to:

- a) the level, consistency and duration of the effect sought in relation to the dose in comparison with a suitable reference product or products, where they exist, and an untreated control;
- b) where relevant, the effect on hygiene, reduction of loss, storage period, in terms of quantity and/or quality, in comparison with an untreated control and, where they exist, a suitable reference product or products.

Where no suitable reference product exists, the efficacy of the biocidal product must be evaluated to determine whether there is a consistent and defined benefit under the normal conditions – including climatic – likely to be encountered in practice in the area of proposed use.

Any unintentional adverse effects must be evaluated on the treated area after use of the biocidal product according to the proposed conditions of use in comparison, where relevant, with an untreated control and/or, where they exist, a suitable reference product or products.

This evaluation will take into consideration and include the following information:

- a) efficacy data;
- b) other relevant information on the biocidal product such as nature of the biocidal product, dose, method of application, number and timing of applications, incompatibility with other types of treatments;
- c) all relevant information on the active micro-organism, including biological properties like mode of action, survival, target organism specificity;
- d) the nature, frequency, level and duration of observed toxic/pathogenic effects on target organism(s) and the environmental (including climatic) conditions that affect them;
- e) the area treated with the biocidal product where toxic/pathogenic effects are

observed;

#### **4.2.2.1.1 Product type**

The product type assigned for the intended use should be verified.

#### **4.2.2.1.2 Target organisms**

The information on target organisms for which the biocidal product is effective should be assessed.

#### **4.2.2.1.3 Mode of action/effect on the target organisms**

The mode of action and the effects on the target organisms should be assessed.

#### **4.2.2.1.4 Area of use/Site of application**

The information on the area of use and the site of application should be assessed.

#### **4.2.2.1.5 Directions of use**

The information on directions of use should be assessed.

#### **4.2.2.2 Assessment of the potential resistance to the biocidal product**

The information on the potential resistance of target organisms to the biocidal product should be assessed.

### **4.3 Part C: Evaluation/conclusion/decision criteria**

#### **4.3.1 Active Micro-organism**

##### **4.3.1.1 Evaluation of the efficacy data**

The data provided should demonstrate basic activity of the active micro-organism against the target organisms, when used in accordance with the intended use. Member States must draw conclusions on the need for further information and/or testing.

##### **4.3.1.2 Evaluation of resistance and resistance management strategies**

The data provided should provide sufficient information to evaluate the possibility of the development by the target organism of resistance or cross-resistance to an active micro-organism. Guidance on the evaluation of resistance can be found in the revised chapter 6.2 of the TNsG on Product Evaluation (33rd CA meeting May 2009). To avoid development of resistance management strategies need to be established.

##### **4.3.1.3 Decision criteria**

No authorisation must be granted if, at any stage in the development of a microbial biocidal product, it becomes apparent, that the active micro-organism is capable of transferring resistance or tolerance genes against antibiotics or disinfectants or if an intrinsic non-transferable resistance against more than one group of antibiotics used in human or animal medicine is shown.

#### **4.3.2 Biocidal Product**

For microbial plant protection products EPPO has published principles of efficacy evaluation for microbial plant protection products (OEPP/EPPO Bulletin (2012) 42 (3), 348–352). These principles may be useful to consider also for microbial biocidal

products. Further information on efficacy evaluation for biocidal products can be found in the TNsG's on product evaluation (available on ECHA website)<sup>29</sup> or in the Biocides Guidance, Volume II Efficacy, Part B Assessment when available. These often do not have specific information on microbial biocidal products, however, in general the same rules for evaluation are applicable for chemical and microbial products.

#### 4.3.2.1 Evaluation of the efficacy data/studies

The data provided should demonstrate sufficient efficacy of the microbial biocidal product against the target organisms, when used in accordance with the intended use. The level, consistency and duration of control or protection or other intended effects must be similar to those resulting from the use of suitable reference products. If no suitable reference product exists, the biocidal product must be shown to give a defined benefit in terms of the level, consistency and duration of control or protection or other intended effects under the animal and human health and environmental (including climatic) conditions in the area of proposed use. The efficacy of a biocidal product should always be assessed with respect to the label claim of the product and the label claims should be relevant with respect to the efficacy of the product. The TNsG on product evaluation (TNsG on Product Evaluation Chapter 7) provides information per PT on evaluation of chemicals which may be useful information for active micro-organisms as well. It specifies what level of efficacy is sufficient per type of use.

#### 4.3.2.2 Performance standards

No authorisation must be granted where the proposed uses include recommendations for the control of or protection against target organisms which are not considered to be harmful<sup>30</sup> on the basis of experience acquired or scientific evidence under normal animal and human health and environmental (including climatic) conditions in the areas of proposed use or where the other intended effects are not considered to be beneficial under those conditions.

Conclusions as to the performance of the preparation must be valid for all areas of the Member State in which it is to be authorised, and for all conditions under which its use is proposed, except where the proposed label specifies that the preparation is intended for use in certain specified circumstances.

#### 4.3.2.3 Evaluation of resistance

The evaluating body must, where relevant, evaluate the possibility of the development by the target organism of resistance or cross-resistance to an active micro-organism in the biocidal product.

If there is evidence of a development of unacceptable tolerance, resistance/cross-resistance of pathogens towards the biocidal product, the Member State must decide if the submitted tolerance or resistance management strategy addresses this adequately and sufficiently.

#### 4.3.2.4 Decision criteria

Authorisation must only be granted if the microbial biocidal product is sufficiently effective.

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<sup>29</sup> <http://echa.europa.eu/guidance-documents/guidance-on-biocides-legislation/biocidal-products-directive>.

<sup>30</sup> Harmful as defined in the Regulation on biocidal products 528/2012/EU Article 3(1)(g).

## 5. Effects on human and animal health

### 5.1 Part A: Information requirements

The information provided on the micro-organism, together with that provided for one or more biocidal products containing the micro-organism must be sufficient to permit a decision whether or not the micro-organism can be included in a Union list of approved active substances. Therefore, toxicological data must allow for an assessment of the potential hazards of the TGAI which may bring risk for humans and animals if exposed to the micro-organism and any relevant metabolites/toxins produced.

#### 5.1.1 Active Micro-organism

The ability of the micro-organism and/or its metabolites and contaminants to cause effects on human and animal health must be carefully assessed. This assessment should be made taking into consideration all data available that would be relevant to address the core and additional data requirements listed in Annex II (title 2) of the BPR. Due to the smaller data package and the fact that suitable test systems are not available for all endpoints it is recognised that it may not always be possible to conclude on the potential to cause adverse effects and on effect levels with the same level of certainty as for chemical substances. Nevertheless, the data must suffice for an understanding of the potential toxicological hazards and risks associated with the micro-organism in order to enable a decision on approval. Any uncertainties noted in the assessment should be indicated and plausible strategies to handle these uncertainties should be discussed.

All effects found during the assessment should be reported. If signs of toxicity, infectivity or pathogenicity are observed, additional testing may be necessary in order to assess the significance of the observed effects, where relevant establish a No Observed Adverse Effect Level (NOAEL) and, if possible, investigate the mechanism involved. For all studies the actual achieved dose must be reported in colony forming units per kg body weight (cfu/kg) and/or in other appropriate units.

For a full evaluation of the potential of the micro-organism to cause toxicity, infectivity and/or pathogenicity, the studies listed in this section should be assessed in combination with information on the biological properties of the micro-organism. Examples of biological properties that can give valuable information on possible health effects of the micro-organism are its:

- life cycle;
- optimal and maximum growth temperature, where a growth temperature below human body temperature can be a sign of a low potential for infectivity;
- other growth requirements, which may indicate a lack of ability to reproduce or grow in the human or animal body;
- production of metabolites/toxins, which may cause toxicity in humans and animals;
- taxonomic group and relationship with pathogenic or toxin-producing species which would require particular attention;
- mode of action, which can give an indication on whether adverse effects are likely to occur in humans and animals;
- target organism(s) and species specificity, where a narrow range of target organisms, especially if taxonomically distant from mammals, could indicate a lower risk of adverse effects in humans and animals.

The expected exposure patterns and levels under the proposed conditions of use should be taken into consideration when deciding on the need for (further) studies or information and the relevance of study findings to the risk assessment.

Whether or not the micro-organism fulfils the criteria for Qualified Presumption of Safety<sup>31,32</sup> (QPS) may also provide useful information.

**NOTE to the reader:** The endpoints described below are numbered in accordance with the BPR, Annex II Information Requirements for Active Substances, Title 2 Micro-organisms, Core data set and additional data set for active substances.

Headings are shown in *italic green font* to distinguish them from the general section numbers of the Guidance document.

## **7. Effect on human and animal health**

Information is required about the micro-organism's potential to cause adverse effects such as infectivity, pathogenicity and/or toxicity as a result of any production of toxins and other relevant metabolites.

### **7.1. Basic information**

This section includes information on effects observed in humans and animals which have been documented for different purposes. This information could include medical records, epidemiological data or case reports described in published literature. Depending on the case, particular consideration may be needed with regards to vulnerable groups of the population such as children and individuals with increased susceptibility due to pre-existing disease, medication, compromised immunity, pregnancy or breast feeding. Information about (e.g.) reported cases and likely exposure should be assessed in order to conclude on opportunistic microorganisms.

#### **7.1.1. Medical data**

Where available, and without prejudice to the provisions of Article 10 of Council Directive 98/24/EC of 7 April 1998<sup>33</sup> and Articles 5 to 17 of Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000<sup>34</sup>, practical data and information relevant to the recognition of the symptoms of infection or pathogenicity and on the effectiveness of first aid and therapeutic measures have to be submitted. Where relevant, methods to kill or attenuate the micro-organism must be provided and the effectiveness of potential antagonists should be investigated and reported.

Information on the effects observed following human exposure, where available and of acceptable quality, is of particular value, in confirming the validity of extrapolations made and conclusions reached with respect to target organs, virulence, and the reversibility of

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<sup>31</sup> Opinion of the Scientific Committee on a request from EFSA on the introduction of a Qualified Assumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. The EFSA Journal (2007) 587, 1-16.

<sup>32</sup> EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2013. Scientific Opinion in the maintenance of the list of QPS biological agents intentionally added to food and feed (2013 update). EFSA Journal 2013;11(11):3449,105 pp. doi:10.2903/j.efsa.2013.3449

<sup>33</sup> Council Directive 98/24/EC of 7 April 1998 on the protection of the health and safety of workers from the risks related to chemical agents at work (fourteenth individual Directive within the meaning of Article 16(1) of Directive 89/391/EEC).

<sup>34</sup> Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC).

adverse effects. Such data can be obtained from reports of accidental or occupational exposure.

### **7.1.2. Medical surveillance on manufacturing plant personnel**

Reports on occupational health surveillance programmes, must be submitted where available.

### **7.1.3. Sensitisation/allergenicity observations**

Available information on sensitisation and allergic reactions observed in workers, including workers in manufacturing plants, agricultural and research workers and others exposed to the micro-organism must be provided. The information should include, observed incidences of hypersensitivity or allergic reactions (dermal and respiratory) as well as details on frequency, level and duration of exposure, symptoms observed and other relevant clinical observations. Information on whether or not workers have been subjected to any allergy tests or interviewed about allergenic symptoms is important.

### **7.1.4. Direct observation, e.g. clinical cases.**

Available reports from the open literature such as epidemiological studies or clinical cases involving the active micro-organism or closely related members of the taxonomic group must be submitted. Such reports are of particular value and should contain complete descriptions of the nature, level and duration of exposure, as well as the clinical symptoms observed, first aid and therapeutic measures applied and any pre-existing disease or compromised immunity in the patients.

## **7.2. Basic studies**

There are currently no OECD guidelines for testing of micro-organisms. Pending such guidelines, the studies required must be generated using available test guidelines for micro-organisms such as USEPA OCSPP Guidelines (formerly known as OPPTS Guidelines).

The observation period recommended in most of the OCSPP guidelines is necessary in order to evaluate infectivity and pathogenicity of the micro-organism considering the incubation time between infection and any clinical symptoms.

It may be relevant to consider, according to the case, both micro-organisms and their spores and other resistant structures. They may co-exist and have different physico-chemical and biological profiles.

### **7.2.1. Sensitisation**

A sensitisation reaction may occur following dermal and/or inhalation exposure.

None of the currently available methods for testing dermal sensitisation are validated for micro-organisms and if conducted, results may be difficult to interpret<sup>35</sup>. The Maximisation test using intradermal injection might be a more reliable method for testing dermal sensitisation than the Buehler or LLNA tests as it ensures bioavailability.

At present, there are no validated test methods for respiratory sensitisation. Evidence that the micro-organism can induce respiratory hypersensitivity is usually based on human experience.

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<sup>35</sup> Similar to the LLNA and Buehler skin sensitisation tests, the in vitro methods have not been validated for the assessment of micro-organisms. Therefore, as neither the in vivo nor in vitro methods have been validated for micro-organisms, the in vitro methods should be considered prior to testing on animals in line with EU legislation that indicates that alternatives to testing on animals should be considered wherever possible.



Due to the unavailability of validated methodology, no studies should be required to address sensitisation and all micro-organisms should be regarded as potential sensitisers until further guidance is available and in the absence of scientific evidence proving otherwise. The following sentence should be included in the label of the product: 'Micro-organisms may have the potential to provoke sensitising reactions'.

### **7.2.2. Acute toxicity, pathogenicity, and infectiveness**

The information provided must be sufficient for an assessment to be made for:

- the toxicity, pathogenicity and infectiveness of the micro-organism after single exposure;
- the time course and characteristics of the effects with full details of behavioural changes and possible gross pathological findings at post-mortem;
- where possible, the mode of toxic action;
- the effects of the micro-organism resulting from exposure via different routes;
- the clearance of the micro-organism.

Acute effects may differ depending on the route of exposure and studies should therefore be performed with oral and pulmonary exposure provided that these routes are relevant with respect to exposure during or after the intended use. For the investigation of infectivity, the intraperitoneal or subcutaneous routes may be more suitable as they ensure systemic exposure to the test organism.

Quantitative information on clearance can be provided by culturing tissue and blood samples at various time points following dosing. Long clearance times are not necessarily indication of harmful effects as long as there's no evidence or reasons to conclude so. But in case of long clearance time an explanation should be given in order to support its acceptability.

#### **7.2.2.1. Acute oral toxicity, pathogenicity and infectiveness**

The acute oral toxicity, pathogenicity and infectiveness of the micro-organism must be reported.

The recommended guideline is OPPTS 885.3050 Acute Oral Toxicity/Pathogenicity.

#### **7.2.2.2. Acute inhalatory toxicity, pathogenicity and infectiveness (ADS)**

The inhalatory toxicity, pathogenicity and infectiveness of the micro-organism must be reported unless inhalation exposure can be excluded. Intratracheal administration could be used to ensure adequate exposure of the test animal to viable micro-organisms. However, inhalation administration may be acceptable if it is demonstrated that a sufficient amount of viable micro-organisms will be inhaled by the test animals, i.e. the dose required by the test guideline.

The recommended guideline is OPPTS 885.3150 Acute Pulmonary Toxicity/ Pathogenicity.

#### **7.2.2.3. Intraperitoneal/subcutaneous single dose (ADS)**

An intraperitoneal/subcutaneous study is required if systemic exposure has not been demonstrated in another study (e.g. acute oral or acute intratracheal). This would be demonstrated by the presence of the micro-organism in several organs and not only at the site of administration.

The recommended guideline is OPPTS 885.3200 Acute Injection Toxicity/Pathogenicity.

### **7.2.3. In vitro genotoxicity testing**

Existing genotoxicity test systems for chemical substances are not validated for testing of micro-organisms. Furthermore, it is unlikely that a whole cell would cause a genotoxic

effect either in mammalian species or in an *in vitro* genotoxicity test system and such studies should therefore not be requested.

If it has been concluded in section 4.1.1 endpoint 3.5 that the micro-organism produces or is likely to produce relevant metabolites, the potential concern for genotoxicity must be addressed. Genotoxicity testing is not necessary if there is sufficient scientific justification to conclude that the metabolites are not relevant.

The relevant metabolite(s) to be tested should, if possible, be investigated in purified form to ensure adequacy of the results. The tests required are thus the same as those recommended for chemical biocides (bacterial assay for gene mutation, test for chromosome aberrations in mammalian cells and test for gene mutation in mammalian cells).

Since micro-organisms may produce a large amount of metabolites, testing of the TGAI or a crude extract taken from an appropriate and justified point in the production process could be considered if the micro-organism is not particularly well known or there is not much information on related strains/species with regard to possible toxins. In such a test, the study design needs to be carefully considered as the concentrations of each component can be expected to be low and a component with a very low genotoxic potential would thus not be detected in the test. It is also necessary to avoid interference by constituents in the test samples. For further guidance, please refer to Part B section 6.2.2 of this Guidance.

#### **7.2.4. Cell culture study**

Cell culture tests must be submitted for intracellularly replicating micro-organisms, such as viruses, viroids or specific bacteria and protozoa, unless the information from sections 1 to 3 clearly demonstrates that the micro-organism does not replicate in warm-blooded organisms.

These studies provide information on the ability of micro-organisms to infect, replicate in, transform or cause toxicity in mammalian cell lines.

The recommended guideline is OPPTS 885.3500 (Cell culture).

#### **7.2.5. Information on short-term toxicity and pathogenicity (ADS)**

The short-term toxicity (minimum 28 days) of the micro-organism should be considered when toxicity, infectivity and/or pathogenicity is observed in one or more acute studies with the micro-organism. Data may also be required to evaluate adverse effects of other components such as microbial contaminants or toxic metabolites.

The study provided must be sufficient to permit the identification of effects following repeated exposure to the micro-organism, and in particular to further establish, or indicate where relevant:

- the toxicity of the TGAI after repeated exposure including where relevant, the NOAEL;
- the infectivity and pathogenicity of the micro-organism after repeated exposure
- target organs;
- the time course and characteristics of the effects with full details of behavioural changes and gross pathological findings at post-mortem;
- the persistence and reversibility of effects observed, following discontinuation of dosing,
- information on the mode of toxic action;
- the clearance of the micro-organism from the main organs as specified in the

study guideline.

The route of administration should be decided based on expert judgement and the effects seen in the acute studies performed.

The recommended guideline is OPPTS 885.3600 Subchronic Toxicity/Pathogenicity.

#### **7.2.5.1. Health effects after repeated inhalatory exposure (ADS)**

Inhalation exposure may occur during application of the biocidal product or, in the case of air-transmitted and spore-forming micro-organisms, also after application in the form of secondary exposure. However, exposure to micro-organisms should be avoided by using respiratory protection since they are considered potentially sensitising by inhalation (please refer to endpoint 7.2.1). Primary exposure is thus not expected to occur.

The possible risk of secondary inhalation exposure must however be addressed and a study in repeated dose inhalation toxicity/infectivity/pathogenicity should be performed if:

- the biological and chemical properties raise a concern for transmission by air, spore formation, and/or dust formation;
- secondary exposure would be expected.

The recommended guideline is OPPTS 885.3600 Subchronic Toxicity/Pathogenicity.

#### **7.2.6. Proposed treatment: first aid measures, medical treatment**

The first aid measures to be used in the event of infection and in the event of contamination of eyes must be provided. Therapeutic regimes for use in the event of ingestion or contamination of eyes and skin must be described in full. This information can be based on practical experience, where available, or on theoretical reasoning.

Information on resistance to antimicrobial agents must be provided under the endpoint 3.6 for active micro-organisms.

### **7.3. Specific toxicity, pathogenicity and infectiveness studies (ADS)**

Specific studies, on toxicity, infectivity and/or pathogenicity must be conducted if the acute or short-term tests under point 7.2 raise a concern for such effects.

The type of study to be performed depends on the effects observed in the acute or short-term studies as well as medical reports of clinical cases or epidemiological studies. Studies to be considered are studies on subchronic toxicity/pathogenicity/infectiveness, chronic toxicity, carcinogenicity, reproductive toxicity or specific studies on one or several relevant toxin(s). Studies in a second species may also be relevant in specific cases, although should be avoided whenever possible.

A study on carcinogenicity may be relevant if:

- proliferative changes are observed in subchronic toxicity studies;
- a virus is shown to be infective in the mammalian cell culture study (see section 5.2.2.2.7 of this Guidance);
- a positive result has been obtained in an *in vivo* genotoxicity study;
- OPPTS guideline 885.3650 Reproductive/fertility effects states that a study on reproductive/fertility effects is required if: significant infectivity of the TGAI is observed in test animals in the subchronic Tier II study (OPPTS 885.3600), and in which no signs of toxicity or pathogenicity were observed; or
- the TGAI is a virus which can persist or replicate in mammalian cell culture lines (OPPTS 885.3500); or

- the TGAI is not amenable to thorough taxonomic classification, but is related to organisms known to be parasitic for mammalian cells; or
- the TGAI or biocidal product is not sufficiently well purified, but it is indicated that it may contain contaminants which are parasitic for mammals.

However, when deciding on the need for specific toxicity studies, the likelihood for exposure should always be taken into consideration<sup>36</sup>. If, for example, a risk assessment is made for a substance that causes a genotoxic effect with a threshold dose and it shows an acceptable risk, a carcinogenicity study would not be required.

For endpoints other than reproductive toxicity, no OCSSP guidelines are available and study design should be decided based on expert judgement and the effects seen in other studies. Before performing further studies, the applicant must seek agreement of the competent authorities on the type of study to be performed.

#### **7.4. Genotoxicity – in vivo studies in somatic cells (ADS)**

*In vivo* studies on genotoxicity in somatic cells are required if positive results have been obtained *in vitro*. The studies could be performed with a crude extract or with a purified metabolite, whichever is relevant and achievable. However, the same material should be tested as that which gave the positive result *in vitro*.

Results from *in vivo* genotoxicity tests should be combined with other available relevant studies to minimise the number of animals used.

The methods and guidance recommended for chemical biocides can be applied (please refer to Volume III B section 6 Mutagenicity).

#### **7.5. Genotoxicity – in vivo studies in germ cells (ADS)**

*In vivo* studies on genotoxicity in germ cells are required if positive results have been obtained in the *in vivo* studies in somatic cells. The studies could be performed with a crude extract or with a purified metabolite, whichever is relevant and achievable. However, the same material should be tested as that which gave the positive result in somatic cells.

The methods and guidance recommended for chemical biocides can be applied (please refer to Volume III B section 6 Mutagenicity).

#### **7.6. Summary of mammalian toxicity, pathogenicity and infectiveness and overall evaluation**

A summary of all data and information must be submitted, with the purpose of establishing the toxicological profile of the TGAI and its potential for infectivity and pathogenicity. The summary should include the following topics:

- the extent, quality and reliability of the data;
- medical reports and clinical cases;
- toxicity, with a description of any effects seen in the studies and whether a NOAEL has been established;
- infectivity, including data on clearance;
- pathogenicity;
- production of toxic metabolites;

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<sup>36</sup> Art 6.2(a) of BPR "Notwithstanding paragraph 1, the applicant need not provide data as part of the dossiers required under points (a) and (b) of paragraph 1 where any of the following applies: (a) the data are not necessary owing to the exposure associated with the proposed uses."

- sensitisation;
- reference values (AOEL, ARfD, ADI) for the active micro-organism and/or one or several relevant metabolite(s) where relevant;
- the need for risk mitigation measures due to the intrinsic properties of the TGAI.

### **7.7. Residues in or on treated articles, food and feedingstuffs (ADS)**

After treatment with the biocidal product, residues may remain in the treated area or on the treated article or may be transferred to food or feedingstuffs. The residues may be viable and/or non-viable.

When the hazard assessment of the TGAI indicates a potential for adverse effects, the information must be provided, to assess the risk for human and animal health, arising from exposure to these residues.

#### **7.7.1. Persistence and likelihood of multiplication in or on treated articles, feedingstuffs or foodstuffs (ADS)**

The persistence of toxic metabolites, where relevant, and the likelihood of persistence and multiplication of the micro-organism in or on treated articles, food or feedingstuffs must be addressed where adverse effects have been observed in sections 7.1 and 7.2 and a reference value has been set for the active micro-organism and/or one or several toxic metabolites.

#### **7.7.2. Further information required (ADS)**

Studies may be required to investigate residues of the following components that may be present in or on treated articles or food or feed:

- the active micro-organism; and/or
- one or several contaminants present in the formulated product; and/or
- one or several relevant metabolites present in the formulated product.

Guidance when such studies are required is given below (points 7.7.2.1 and 7.7.2.2).

##### **7.7.2.1. Non-viable residues (ADS)**

Non-viable residues could be non-viable micro-organisms or metabolites/toxins produced by the active micro-organism either during fermentation or during growth of the active micro-organism after application.

Information on levels of non-viable residues in or on treated articles, food or feed is required when the following applies:

- relevant toxic metabolites or other chemical substances of concern are present in the product;
- relevant toxic metabolites are produced by the micro-organism in or on treated articles, food or feed;
- the non-viable residues are expected to be present at levels that cannot be considered to be negligible.

The levels of non-viable residues could be estimated based on the information provided under point 7.7.1 in addition to information on:

- the production of toxic metabolites on the treatment site;
- the sensitivity to degrading enzymes or other substances that can affect the stability of the metabolite.

If significant exposure to quantities of relevant non-viable residues is expected, full experimental residue data as provided for in Annex II, Title 1, endpoint 8.16 for chemical active substances of the BPR must be submitted.

Before performing such studies, the applicant must seek agreement of the competent authorities on the type of study to be performed.

Where applicable, a detailed justification for non-submission of non-viable residue data must be submitted.

#### **7.7.2.2. Viable residues (ADS)**

Viable residues could be residues of the active micro-organism or of contaminants in the biocidal product that remains in or on treated articles, food or feedingstuffs.

A study on levels of viable residues is required when any of the following applies:

- adverse effects have been observed in points 7.1 and 7.2 and a reference value has been set for the active micro-organism;
- based on the information provided in point 7.7.1, the viable residues are expected to be present at levels that cannot be considered as negligible.

The methods for detection and quantification of the viable residues should be the most relevant and suitable methods available. This should be decided on a case-by-case basis. Please refer to section 4.2.

If significant quantities of viable residues are expected and a hazard cannot be excluded the competent authorities may require studies similar to those provided for in Annex II, Title 1, point 8.16 of the BPR for chemical active substances.

Before performing such studies, the applicant must seek agreement of the competent authorities on the type of study to be performed.

Where applicable, a detailed justification for non-submission of non-viable residue data must be submitted.

#### **7.8. Summary and evaluation of residues in or on treated articles, food and feedingstuffs (ADS)**

A summary of all data and information provided under point 7.7 above, must be submitted, where applicable. The summary should include:

- a detailed and critical assessment of data, and a conclusion on whether or not exposure to residues is likely to be of concern;
- a discussion on the extent, quality and reliability of the data;
- a conclusion on the need for reference values (MRLs) for viable and non-viable residues.

### **5.1.2 Biocidal Product**

The purpose of the studies on the biocidal product is to investigate the potential for adverse effects caused by contaminants, fermentation residues and co-formulants. It must be clear that the data provided in section 6.1.1 of this document actually represent the TGAI used in the biocidal product. Therefore, a detailed description (specification) of the material used must be provided.


Attention should be paid to combined effects of the active micro-organism, co-formulants and contaminants, which may be synergistic, antagonistic, negligible or of other kind. The section on combined exposure included in the general guidance for biocides could be consulted for this assessment (section 4.4 of the *Biocides Guidance Volume III Human Health, Part B Risk Assessment*).

The studies performed on the biocidal product should be sufficient to conclude on the acute toxicity, irritation and sensitisation potential of the biocidal product as laid down in Annex II, Title 2 of the BPR.

The tests submitted must be conducted using the representative product and the information provided must be sufficient to permit an assessment to be made of the identification of:

- the toxicity of the biocidal product following a single exposure;
- toxicity of the biocidal product relative to the TGAI;
- the time course and characteristics of the effect with full details of behavioural changes and possible gross pathological findings at post-mortem;
- where possible the mode of toxic action;
- the hazard associated with the different routes of exposure.

While the emphasis must be on estimating the toxicity ranges involved, the information submitted must also permit the biocidal product to be classified in accordance with Regulation (EC) No 1272/2008 (CLP). The information generated through acute toxicity testing is of particular value in assessing hazards likely to arise in accident situations.

 **NOTE to the reader:** The endpoints described below are numbered in accordance with the BPR Annex III Information Requirements for Biocidal Products, Title 2 Micro-organisms, Core data set and additional data set for active substances.

Headings are shown in *italic green font* to distinguish them from the general section numbers of the Guidance document.

## **8. Toxicological profile for humans and animals**

The fulfilment of the information requirements described should be achieved, whenever possible, via testing methods not involving animals.

### **8.1. Skin corrosion or skin irritation**

The purpose of the test is to provide information on the potential for skin irritation of the biocidal product including any reversibility of the effects observed. Testing on the product does not need to be conducted if:

- a conclusion on the irritating potential of the TGAI can be made based on existing information, e.g. published research available in the open literature;
- there are valid data available on each of the non-active ingredients (co-formulants) in the mixture to decide on classification of the mixture according to the rules laid down in Regulation (EC) No 1907/2006 (REACH) and Regulation (EC) No 1272/2008 (CLP);
- synergistic effects between any of the components are not expected.

The test should be performed according to a recognised guideline such as the OECD Guidelines for the Testing of Chemicals. For the selection of the test method, the *Guidance on the Biocidal Product Regulation, Part A Information requirements for chemicals*<sup>37</sup> applies.

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<sup>37</sup> Available at: <http://echa.europa.eu/web/guest/guidance-documents/guidance-on-biocides-legislation>.

## 8.2. Eye irritation

The purpose of the test is to provide information on the potential for eye irritation of the biocidal product, including any reversibility of the effects observed. Testing on the product does not need to be conducted if:

- a conclusion on the irritating potential of the TGAI can be made based on existing information, e.g. published research available in the open literature; and
- there are valid data available on each of the non-active ingredients (co-formulants) in the mixture to allow classification for skin and eye irritation according to the rules laid down in Regulation (EC) No 1907/2006 (REACH) and Regulation (EC) No 1272/2008 (CLP); and
- synergistic effects between any of the components are not expected.

The test should be performed according to a recognised guideline such as the OECD Guidelines for the Testing of Chemicals. For the selection of the test method, the *Guidance on the Biocidal Product Regulation, Part A Information requirements for chemicals* applies.

## 8.3. Skin sensitisation

All micro-organisms should be regarded as potential sensitisers, until adequate methods or further guidance are available. The product should thus be labelled with the phrase "Contains (name of micro-organism). Micro-organisms may have the potential to provoke sensitising reactions".

Therefore, testing on the product does not need to be conducted. For classification of the mixture according to the rules laid down in Regulation (EC) No 1272/2008 (CLP), data available on each of the chemical components in the mixture can be used provided that no synergistic effects between any of the components are expected.

## 8.4. Respiratory sensitisation (ADS)

All micro-organisms should be regarded as potential sensitisers until adequate methods or further guidance are available. The product should thus be labelled with the phrase "Contains (name of micro-organism). Micro-organisms may have the potential to provoke sensitising reactions".

Therefore, testing on the product does not need to be conducted. For classification of the mixture according to the rules laid down in Regulation (EC) No 1272/2008 (CLP), data available on each of the chemical components in the product can be used provided that synergistic effects between any of the components are not expected.

## 8.5. Acute toxicity

Testing of the biocidal product does not need to be conducted if there are valid data available for each of the components in the product sufficient to allow classification of the product according to the rules laid down in Regulation (EC) No 1272/2008 (CLP), and synergistic effects between any of the components are not expected.

### 8.5.1. Oral

An acute oral toxicity test must be carried out unless the applicant can justify in accordance with Article 21(1) of the BPR that it would be scientifically unnecessary or technically not feasible to conduct the test.

The test should be performed according to a recognised guideline such as the OECD Guidelines for the Testing of Chemicals.

### 8.5.2. Inhalation

An acute inhalation toxicity test must be carried out if:



- one or several co-formulants are volatile (a volatile substance has vapour pressure  $> 1 \times 10^{-2}$  Pa at 20 °C);
- the product is a powder or is applied in a manner that generates exposure to aerosols, particles or droplets of an inhalable size (MMAD  $< 50 \mu\text{m}$ );

unless the applicant can justify in accordance with Article 21(1) of the BPR that it would be scientifically unnecessary or technically not feasible to conduct the test.

The test should be performed according to a recognised guideline such as the OECD Guidelines for the Testing of Chemicals.

### **8.5.3. Dermal**

An acute dermal toxicity test must be carried out unless the applicant can justify in accordance with Article 21(1) of the BPR that it would be scientifically unnecessary to conduct the test or technically not feasible.

The test should be performed according to a recognised guideline such as the OECD Guidelines for the Testing of Chemicals. For the selection of the test method, the Guidance on the Biocidal Product Regulation, Part A Information requirements for chemicals<sup>38</sup> applies.

### **8.5.4. Additional acute toxicity studies**

In certain cases it may be necessary to carry out the studies as referred to under the endpoints 8.1 to 8.5.3 for biocidal product for a combination of biocidal products where the product label includes requirements for use of the biocidal product with other biocidal products and/or with adjuvants as a tank mix.

Decisions as to the need for supplementary studies must be made on a case-by-case basis, taking into account the results of the acute toxicity studies of the individual biocidal products, the possibility for exposure to the combination of the products concerned and available information or practical experience with the products concerned or similar products. In general, additional tests involving vertebrate animals should be avoided whenever possible.

### **8.6. Information on dermal absorption, if required**

Micro-organisms are not expected to penetrate intact skin, thus no dermal absorption data is required on the active micro-organism.

Dermal absorption data is required for toxic metabolites and/or co-formulants present in the biocidal product if it has been concluded in previous sections that effects of these substance(s) need to be considered in the risk assessment.

If no specific absorption data is available, a default assumption should be made in accordance with guidance for chemical substances.

In case dermal absorption studies are required, an *in vitro* study according to OECD TG 428 should be conducted rather than *in vivo* tests.

### **8.7. Available toxicological data relating to:**

- **non-active substance(s) (i.e. substance(s) of concern), or**
- **a mixture that a substance(s) of concern is a component of**

Annex III, Title 2, 8.7 Column 1 states: *If insufficient data are available for a non-active substance(s) and cannot be inferred through read-across or other accepted non-testing*

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<sup>38</sup> Available at: <http://echa.europa.eu/web/guest/guidance-documents/guidance-on-biocides-legislation>.

*approaches, targeted test(s) described in Annex II, shall be carried out for the substance(s) of concern or a mixture that a substance(s) of concern is a component of.*

If information available, for instance in the safety data sheet, indicate that a substance may cause certain toxic effects, targeted testing of the product for that effect may be required.

Annex III, Title 2, 8.7 Column 3 states: *Testing on the product/mixture does not need to be conducted if:*

- *there are valid data available on each of the components in the mixture sufficient to allow classification of the mixture according to the rules laid down in Directive 1999/45/EC and Regulation (EC) No 1272/2008 (CLP), and synergistic effects between any of the components are not expected.*

### **8.8. Supplementary studies for combinations of biocidal products**

Annex III, Title 2, 8.8 Column 1 states: *For biocidal products that are intended to be authorised for use with other biocidal products, the risks to humans and the environment arising from the use of these product combinations shall be assessed. As an alternative to acute toxicity studies calculations can be used. In some cases, for example, where there is no valid data available of the kind set out in column 3, this may require a limited number of acute toxicity studies to be carried using combinations of the products.*

Information should be provided if applicable.

Annex III, Title 2, 8.8 Column 3 states: *Testing on the mixture of products does not need to be conducted if:*

*there are valid data available on each of the components in the mixture to allow classification of the mixture according to the rules laid down in Directive 1999/45/EC, Regulation (EC) No 1907/2006 (REACH) and Regulation (EC) No 1272/2008 (CLP), and synergistic effects between any of the components are not expected*

### **8.9. Residues in or on treated articles, food and feedingstuffs (ADS)**

The same provisions as detailed under the endpoint 7.7 for active micro-organisms, apply. The information required according to this section has to be provided unless it is possible to extrapolate the residue data of the biocidal product on the basis of the data available for the micro-organism.

Special attention should be paid towards the influence on the residue behaviour of the micro-organism and its relevant metabolites.

## **5.2 Part B: Hazard, Exposure and risk assessment**

### **5.2.1 General aspects**

Hazards arising from micro-organisms are not necessarily of the same nature as those presented by chemicals. Micro-organisms are unlikely to be toxic in themselves but may produce toxic metabolites during fermentation and/or in contact with the target organism. They may also have a potential to cause sensitising reactions and non-specific effects such as inflammation responses after inhalation exposure. Micro-organisms also have the capacity to replicate and their ability to cause infection or pathogenicity in humans and animals must be carefully assessed.

The metabolites of concern (i.e. toxins) produced during fermentation and present in the biocidal product may be considered to be present at concentrations most relevant for risk assessment. This means that data on the TGAI/product regarding metabolites is relevant for users of the product at application but not necessarily for consumers in

contact with treated articles or food/feed as the fermentation metabolites are not necessarily the same as those produced at the site of application, or the concentrations may differ. If relevant metabolites play a major role in the mode of action of the active micro-organisms, their level of occurrence at the site of application needs to be estimated and the exposure of consumers addressed.

Besides metabolites/toxins, toxicologically relevant compounds in the biocidal product may include residual growth medium, contaminants and co-formulants. These are tested in the product studies performed.

It may be relevant to consider, according to the case, both micro-organisms and their spores and other resistant structures. They may co-exist and have different physico-chemical and biological profiles.

The exposure assessment should include both primary and secondary exposure to humans under proposed conditions of use. The exposure assessment to a micro-organism should in general be qualitative or semi-quantitative because there are normally no reference values to which exposure levels could be compared.

The exposure assessment should also include any relevant metabolites/toxins present in the product or produced in contact with the target organism. If quantitative data is available for a relevant metabolite, the exposure may be assessed in the same way as for chemical biocides.

## 5.2.2 Hazard assessment

### 5.2.2.1 Effects on human and animal health

The purpose of the human and animal health assessment is to investigate the toxicity, infectivity and pathogenicity potential of the micro-organism as well as the toxicity of any metabolites or other chemical components present in the TGAI.

Infectivity refers to the ability of the micro-organism to grow and proliferate in the body of the host, in this case humans or animals. The biological properties of the micro-organism such as the optimal and maximum growth temperature can give indications on the organism's potential for infectivity but this endpoint is primarily assessed by the investigation of clearance from the body in the animal studies performed. A non-infective micro-organism is cleared from the body whereas an increase in cfu count (or other relevant unit) over time would be seen for an infective micro-organism<sup>39</sup>.

Pathogenicity is the ability of the micro-organism to grow and proliferate in the host but also to cause disease in the host. Signs of pathogenicity in an animal study could be elevated body temperature, histopathological findings and unexpected mortality. Micro-organisms that are pathogenic to mammals must not be approved for use in biocidal products. However, some micro-organisms that are normally not pathogenic may be opportunistic pathogens and cause infections in immunocompromised persons. Such information could be obtained from clinical case reports of infections in humans available in the public literature.

#### 5.2.2.1.1 Medical data

Information on symptoms of infection or pathogenicity caused by the micro-organism may be available from medical reports or from case reports described in the open literature. This could refer to systemic infections or local infections such as eye infections.

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<sup>39</sup> Note that long clearance time is not necessarily related to harmful effects. See section 5.1.1, endpoint 7.2.2.

It is important to note whether or not the infections occurred in the presence of an intact immune defence.

Information on first aid and therapeutic measures are of high importance to the treatment of the infections.

#### **5.2.2.1.2 Medical surveillance on manufacturing plant personnel**

Reports on occupational health surveillance programmes should include detailed information on the design of the programme as well as on frequency, level and duration of exposure to the micro-organism. Preferably, these reports must include data from persons exposed in manufacturing plants or after application of the micro-organism (e.g. in efficacy trials).

#### **5.2.2.1.3 Sensitisation/allergenicity observations**

Observations of sensitisation reactions in humans may be reported in exposure studies available in the open literature or they may be documented in medical records held by the manufacturer of the TGAI or any pest control companies using products containing the TGAI. These records provide useful information, particularly as there are no validated methods for testing of sensitisation in animals. The mechanisms involved in dermal sensitisation are not the same as those involved in sensitisation by inhalation and thus one micro-organism may cause sensitisation by one of the routes but not the other. However, due to the lack of suitable testing methods, all micro-organisms are considered as potentially sensitising by both the dermal and the inhalation route until further guidance becomes available or scientific evidence shows otherwise. The following sentence should therefore be included on the label of the product: 'Micro-organisms may have the potential to provoke sensitising reactions'<sup>40</sup>. In case there is clear evidence in literature that the microorganism is respiratory sensitiser, classification applies instead of the warning phrase.

#### **5.2.2.1.4 Direct observation, e.g. clinical cases.**

Clinical case reports and epidemiological studies of the active micro-organism or of any taxonomically related strains and species should be considered to assess whether the active micro-organism is known to cause infection and pathogenicity in humans. If the micro-organism considered in the studies is a different species than the micro-organism being assessed, it is important to clarify what distinguishes the two (i.e. the active micro-organism and the pathogenic species object of the studies) and whether it is likely that the active micro-organism could exhibit the same properties. For such an analysis, information on the biological properties of the micro-organism such as growth requirements and the presence of genes encoding known toxins may be useful. If the pathogenic species requires significantly different growth conditions or is taxonomically not closely related, that could be indications of a lower risk of pathogenicity associated with the active micro-organism.

If the micro-organism is ubiquitous in nature it may be identified as being present in infections following traumatic wounding. In such cases it has to be confirmed whether or not the micro-organism was causative of the infection. Reports of infective activity where humans have been directly exposed (i.e. spraying preparations) to the active micro-organisms should be taken into account. A reasoning whether or not there is a risk that the active micro-organism may cause true infectious disease in mammals, including humans, is necessary.

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<sup>40</sup> Discussed at CTGB workshop on the toxicological risk assessment of pesticides using micro-organisms (November 2015).

## 5.2.2.2 Basic studies

### 5.2.2.2.1 Sensitisation

There are currently no validated methods for investigating sensitisation by micro-organisms and consequently no studies can be required. However, if a dermal sensitisation test has been conducted with the TGAI the results, either positive or negative, should be interpreted with caution since the current dermal sensitisation tests are not validated for microorganisms. These tests are optimised for testing of single substances for which the test concentration can be maximised and controlled. This is difficult to achieve when testing complex mixtures containing many different compounds present at low concentrations. The possibility of a sensitising metabolite and/or false positive finding should also be taken into account.

At present, there are also no validated test methods for respiratory sensitisation and consequently no studies can be required.

Therefore, all micro-organisms should be regarded as potential sensitisers, until further guidance is available or scientific evidence shows otherwise.

### 5.2.2.2.2 Acute toxicity, pathogenicity, and infectiveness

The information generated through acute toxicity, pathogenicity and infectiveness testing is of particular value to the assessment of hazards that are likely to arise in accidental situations with high exposure to the TGAI. These studies are also useful to decide on the need for further studies and on the type of studies that should be performed.

When toxic effects are observed, an attempt to investigate the cause of the toxicity should be made. The micro-organism as such is unlikely to cause direct toxic effects, however indirect effects could possibly be expected, for example, after intratracheal exposure when the presence of foreign material in the respiratory system may evoke an inflammation reaction. Direct toxic effects would more likely be caused by a metabolite/toxin produced by the active micro-organism.

### 5.2.2.2.3 Acute oral toxicity, pathogenicity and infectiveness

Studies by the oral route may indicate if there is a risk for toxic effects following exposure resulting from hand to mouth transfer of residues remaining on treated surfaces or resulting from inhalation exposure when residues can be transported via the mucociliary system and swallowed. In contrast to the other types of exposure routes considered in this section, the micro-organism is via this route exposed to the conditions of the oral cavity, the gastro-intestinal tract and the first-pass mechanism of the liver before entering the systemic circulation.

### 5.2.2.2.4 Acute inhalatory toxicity, pathogenicity and infectiveness (ADS)

Pulmonary toxicity/infectivity/pathogenicity can be tested through inhalation exposure or intratracheal exposure. Intratracheal administration could be used to ensure adequate exposure of the test animal to micro-organisms. Generally, the concentration of micro-organism in the atmosphere becomes too low and the particle size distribution too high when administered via inhalation. Furthermore, the viability of the micro-organism is often compromised due to shear forces from nebulisation. Most vegetative microbes, particularly Gram-negatives, suffer considerable damage (about 95% are killed) while gram positives are less sensitive and most spores survive. Fungi are difficult to get into a respirable aerosols without significant loss in viability because of their size. Due to these considerations inhalation exposure is normally not recommended for micro-organisms.

In order to exclude non-specific effects as a result of the intrusion, rather than the TGAI, the use of a sham control could be considered.

#### 5.2.2.2.5 Intraperitoneal/subcutaneous single dose (ADS)

The intraperitoneal/subcutaneous test is considered a highly sensitive assay to investigate infectivity since the way of administration ensures exposure of the test animals to the micro-organism. Intraperitoneal injection is the preferred administration way, however considering that the temperature of the skin is lower than the body temperature, subcutaneous injection may in some situations be more relevant. This could be the case if the maximum temperature for growth and multiplication is below 37°C as the micro-organism would then be more likely to cause infections in the skin rather than deep tissue infections.

#### 5.2.2.2.6 *In vitro* genotoxicity testing

Existing genotoxicity test systems for chemical substances are not validated for testing of whole micro-organisms. Furthermore, it is considered unlikely that a whole cell would cause a genotoxic effect either in mammalian species or in an *in vitro* genotoxicity test system and such studies should therefore not be requested.

*In vitro* genotoxicity testing may however be relevant for metabolites. These could be tested in purified form using the same test methods as for chemical biocides. However, since micro-organisms may produce a large amount of metabolites, testing of a crude extract (i.e. the chemical constituents of the TGAI with cell walls etc., removed) could be considered. In such a test, the study design needs to be carefully considered as the concentrations of each component can be expected to be low and a component with a low genotoxic potential would thus not be detected in the test.

When performing genotoxicity studies with a crude extract (see under 7.2.3 in sub-section 6.1.1 for when such studies should be used) it is necessary to avoid interference by constituents in the test samples (examples by J.T. MacGregor, 2005<sup>41</sup>):

- interference from toxic components;
- provision of nutrients by lysates (e.g. histidine, which would allow growth of the auxotrophic tester strains in the Ames *Salmonella* assay);
- growth factors that may produce abnormal growth, growth inhibition, or DNA synthesis (e.g. erythropoietin which causes micronuclei in bone marrow *via* induction of abnormal cell proliferation; lectins that may stimulate DNA synthesis in *in vitro* mammalian cell tests);
- enzymatic activity that could mimic endogenous activity in the test organism (e.g., kinase or phosphokinase activity in the TK<sup>+/-</sup> or HPRT assays);
- the occurrence of potentially active constituents as bound or complexed forms (e.g. as glycosides or other conjugates, or bound to macromolecular constituents);
- intracellular molecules with nuclease or proteolytic activity from *in vitro* lysates that would not normally have access to mammalian cells *in vivo*.

It is important that a flexible approach is adopted with modifications of standard test protocols as necessary to adjust for the factors listed above. Selection of the methodology for further testing depends on how the results are interpreted at each

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<sup>41</sup> James T. MacGregor. Genetic Toxicity Assessment of Microbial Pesticides: Needs and Recommended Approaches. Report to OECD. December 2005.

stage. This is further described in the OECD report *Genetic Toxicity Assessment of Microbial Pesticides: Needs and Recommended Approaches* (J.T. MacGregor, 2005).

In the case of a virus the risk of insertion mutagenesis in mammal cells and the risk of carcinogenicity has to be discussed.

#### **5.2.2.2.7 Cell culture study**

A cell culture study in a recognised mammalian cell line gives information on the ability of a viral pest control agent to infect, replicate in, transform or cause toxicity in the cell system.

The study should be performed in human cell or tissue cultures of different organs. Selection can be based on expected target organs after infection. If human cell or tissue cultures of specific organs are not available, other mammalian cell and tissue cultures can be used.

OPPTS guideline 885.3500 states that if the data show that the viral pest control agent preparation is toxic to any of the test cell cultures, but does not infect, replicate in or transform any of the cell cultures, further information may be required to identify the toxic components of the preparation. Moreover, an acute toxicity study may be required with the toxic components.

If the viral pest control agent infects any of the test cell cultures, reproductive and fertility effects, carcinogenicity, immunodeficiency, and primate infectivity/pathogenicity studies may be required (see section 6.2.2.3).

#### **5.2.2.2.8 Information on short-term toxicity and pathogenicity (ADS)**

While the acute toxicity studies provide information on the toxic/infective/pathogenic properties of the TGAI after a single high dose, they are not suitable for use in a risk assessment which is made to ensure that there is no risk for effects following repeated exposure to the TGAI. Therefore, if adverse effects have been observed after acute exposure, further testing may be necessary to clarify the nature and severity of effects that may result from repeated administration of the TGAI. Moreover, an incomplete or very slow clearance rate may indicate an infectious potential and thus trigger further testing to exclude pathogenicity (when no clinical signs or effects are detected, long clearance time can be acceptable but needs to be justified). A need for further investigations of effects following repeated exposure may also be triggered by information available in medical records, published reports of clinical case or epidemiological studies.

The duration of the short-term study can be decided based on the clearance data and the effects seen in the acute toxicity study but should normally be 90 days.

#### **5.2.2.2.9 Health effects after repeated inhalatory exposure (ADS)**

In case the biological and chemical properties raise a concern for transmission by air, by spore formation, and/or by dust formation, further investigations of effects resulting from repeated exposure via inhalation may be needed in case secondary exposure can be expected.

#### **5.2.2.2.10 Specific toxicity, pathogenicity and infectiveness studies (ADS)**

There may be medical reports of clinical cases, epidemiological studies or toxic, infective or pathogenic effects observed in the acute or short-term studies that raise concerns and require further investigation.

If toxic effects are reported or observed, depending on the type of effects subchronic or chronic toxicity studies may be required and may need to include specific investigations of endpoints such as neurotoxicity, carcinogenicity, reproductive toxicity or

immunotoxicity. Specific studies may also be required in order to set a reference value for use in risk assessment<sup>42</sup>.

Signs of infectivity and pathogenicity need to be thoroughly investigated. In general a non-infective micro-organism is cleared from the body whereas an increase in cfu count (or other relevant unit) over time would be seen for an infective micro-organism. With regard to what organs should be analysed for their microbial content, no deviation from the available test guidelines should be allowed. In case the clearance is not complete by the end of the study the applicant should give an explanation on this effect. A micro-organism that is infective (please refer to definitions in section 2.1) should be investigated for reproductive effects since the risk of an infection during pregnancy causing harm to the unborn child must be addressed. Organisms infective to human cell lines may also need further investigations.

#### **5.2.2.2.11 Genotoxicity - in vivo studies in somatic cells (ADS)**

If a positive result has been obtained in an *in vitro* study with a purified metabolite, the metabolite needs to be investigated for *in vivo* genotoxicity in somatic cells.

If a positive result has been obtained in an *in vitro* study with a crude extract, the extract needs to be investigated for *in vivo* genotoxicity in somatic cells.

A crude extract can also be used for unknown micro-organisms or when little information is available on toxins from related strains/species.

The methods recommended are the same as for chemical biocides.

To be reminded that results from *in vivo* genotoxicity studies should be combined, whenever possible, with other available studies in order to minimise the number of animals involved.

#### **5.2.2.2.12 Genotoxicity - in vivo studies in germ cells (ADS)**

If a positive result has been obtained in an *in vitro* study with a purified metabolite, the metabolite needs to be investigated for *in vivo* genotoxicity in germ cells.

If a positive result has been obtained in an *in vitro* study with a crude extract, the extract needs to be investigated for *in vivo* genotoxicity in germ cells.

The methods recommended are the same as for chemical biocides.

#### **5.2.2.2.13 Residues in or on treated articles, food and feedingstuffs (ADS)**

After treatment with the biocidal product, residues may remain on the treated article or may be transferred to food or feedingstuffs. The residues may be viable and/or non-viable and can be a source of secondary exposure to human. There is currently no guidance available on the transfer of residues to food and feedingstuffs. However, guidance documents being developed within the Ad hoc Working Group on the Assessment of Residue Transfer to Food<sup>43</sup> may be consulted if considered relevant.

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<sup>42</sup> Please note that if these kinds of tests are considered necessary the registrant must submit an inquiry to ECHA in accordance with Article 62(2) of the BPR to determine if these tests are already available. In addition, in the context of the pre-submission consultation, the applicant can discuss with the evaluating competent authority the tests he intends to carry out.

<sup>43</sup> <http://echa.europa.eu/about-us/who-we-are/biocidal-products-committee/working-groups/assessment-of-residue-transfer-to-food>.



#### **5.2.2.2.14 Persistence and likelihood of multiplication in or on treated articles, feedingstuffs or foodstuffs (ADS)**

Information on the persistence and likelihood of multiplication in or on treated articles, feedingstuffs or foodstuffs could be obtained from the Fate and behaviour section or could be estimated based on the proposed uses and conditions of use in combination with data on biological properties such as:

- growth requirements;
- development stages/life cycle under typical environmental conditions (*i.e.* in or on the treated area);
- ecological niche, host specificity and mode of action;
- sensitivity to factors such as temperature, UV light, pH and the presence of competing micro-organisms;

or any other relevant information available. Information on practical experience can be used to support the estimation. The reasoning should be described in detail.

At present, guidance on secondary metabolites produced by entomopathogens is being developed.

#### **5.2.2.3 Further information required (ADS)**

##### **5.2.2.3.1 Non-viable residues (ADS)**

Non-viable residues could be metabolites/toxins produced by the active micro-organism during fermentation, in which case they would be present during application of the product, or during growth of the active micro-organism, in which case they would be produced on the treated area. The metabolites produced during fermentation and present in the biocidal product are not necessarily the same as those produced at the site of application, or the concentrations may differ. Presence of non viable residues in situ should be addressed or considered.

##### **5.2.2.3.2 Viable residues (ADS)**

Viable residues could be spores or active forms of the active micro-organism or of contaminants present in the biocidal product that remains in or on treated surfaces, food or feedingstuffs after application of the biocidal product. Although strictly speaking they are not living, viruses, viroids and transposones should be considered as viable residues since they are capable of transferring genetic material.

#### **5.2.3 Exposure assessment**

##### **5.2.3.1 Primary and secondary exposure of humans**

In exposure assessments, a distinction is made between primary and secondary exposure. Primary exposure refers to the exposure occurring during application of the product whereas secondary exposure refers to exposure occurring after the product has been applied.

Primary exposure includes all tasks related to application, for example, delivery and handling of products, dilution of concentrates and/or loading of product, application by hand, by hand-held tool, by dipping and by spraying.

All exposure which is not primary exposure is considered as secondary exposure, for example, contact with treated areas by others than the person applying the product.

Both primary and secondary exposure of humans that is likely to occur under the proposed conditions of use, must be assessed. The assessment should take into consideration the active micro-organism, and/or exposure to toxicologically relevant compounds in the biocidal product. Toxicologically relevant compounds in the biocidal product may include metabolites/toxins, residual growth medium, contaminants and co-formulants (see section 3).

### 5.2.3.2 Different routes of exposure

Exposure may occur via any or several of the main routes, i.e. the oral, dermal and inhalation routes, and it can be to the active micro-organism and/or spores. The route being the predominant route of exposure depends on the intended use and the physical properties of the product. If the product is applied by spraying, the significant route of exposure is likely via inhalation. However, if the equipment used for application generates droplets of a non-respirable size, the exposure will rather be via the oral route since the product inhaled will be swallowed after being mucociliarly transported from the respiratory tract. Moreover, the time taken for the spray to settle varies with droplet size and consequently also the potential for dermal exposure. It is also important to note that the significant routes of exposure may differ depending on the exposure category. Whereas a user may primarily be exposed via inhalation, residents may primarily be exposed via dermal contact with treated areas. Unintentional hand to mouth transfer may need to be considered in exceptional cases. The intact micro-organism is not expected to be absorbed through the skin but dermal absorption of metabolites may occur, if present after application. Therefore, the need to consider dermal exposure depends on information on metabolite production in section 4. If no specific absorption data is available for the metabolite, the default assumption would be to consider a 75% absorption. The exposure can then be calculated from this data in combination with residue data.

The exposure during application can be reduced if the user is wearing personal protective equipment (PPE) such as protective clothing, gloves, face mask and respiratory protection. However, since consumers do not have easy access to this type of equipment, refinement of exposure based on the use of PPE can only be applied to exposure assessments of professional users, i.e. a user which is subject to national worker protection legislation and has knowledge and experience of frequent handling of biocidal products. The different user categories are further described in [Guidance on the BPR: Volume III Human Health, Part B Assessment](#).

In the absence of appropriate test methods (see section 6.2.2.2.1 above) all micro-organisms are currently assumed to have the potential to cause sensitisation reactions in humans. Therefore, in the assessment of primary exposure, the user may be assumed to wear protective clothing (PPE). To be noted that with regard to PPE there is no harmonized approach possible due to national requirements. Some Member States require respiratory protective equipment (RPE) for certain types of products (e.g. mixing and loading of powders) or type of application (indoor) while other Member States always prescribe RPE for all micro-organisms (hazard approach). Other types of risk mitigation measures may be considered where relevant (e.g. packaging; spot treatment at inaccessible locations).

### 5.2.3.3 Exposure of animals

In case exposure of livestock and/or pets is anticipated, this exposure also needs to be addressed. Such assessment can start from the guidance given in the document.

There is currently no guidance available, neither a specific guidance for micro-organisms nor a general guidance for biocides. However, guidance documents being developed

within the BPC Ad hoc Working Group on the Assessment of Residue Transfer to Food<sup>44</sup> related to chemical substances may be consulted if considered relevant.

#### 5.2.3.4 Assessment of primary exposure

The exposure assessment to a micro-organism should in general be qualitative or semi-quantitative because there are normally no reference values to which exposure levels could be compared.

Quantitative information, wherever available, should be used to describe the levels of exposure. Ideally, realistic data on exposure levels, i.e. measured data from field studies should be used for the assessment of primary exposure. If such data are not available, an estimate of the expected exposure level can be obtained using a calculation model, although the current models<sup>45</sup> generally used for biocides in exposure assessments of certain scenarios may not be applicable for micro-organisms. Moreover, since micro-organisms may produce secondary metabolites such as toxins when in contact with the target organism, the potential for this type of exposure should be discussed and, if possible, assessed based on the information in section 4 of this guidance. If quantitative data would be available for a secondary metabolite (the amount present in the product or present on the treated area), the exposure may be assessed in the same way as for chemical biocides.

The following information which is required in accordance with Annex III of the BPR should be considered along with other relevant information for the exposure assessment:

- composition of the product;
- physical form of the product (e.g. wettable powder, granular concentrate, soluble concentrate);
- size, design and type of packaging, - depending on the design, spillage and thus accidental exposure may be reduced;
- product type and the intended use of the product;
- method of application including mixing of the biocidal product;
- maximum application rate;
- number and timing of applications;
- category of user (i.e. professional user or consumer);
- for refinement of exposure assessments, exposure reduction measures such as use of personal protective equipment should be considered.

Exposure assessments must be made for each type of application method and equipment (including the types and sizes of the containers used) In case of concomitant use of other biocidal products containing the same micro-organism in the area of the envisaged use, aggregated exposure should be considered.

#### 5.2.3.5 Assessment of secondary exposure

The possibility of secondary exposure such as exposure of bystanders or residents, visitors or animals staying in treated areas, needs to be assessed. Similarly to primary

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<sup>44</sup> <http://echa.europa.eu/about-us/who-we-are/biocidal-products-committee/working-groups/assessment-of-residue-transfer-to-food>.

<sup>45</sup> Guidance on the BPR: Volume III Human Health, Part B Assessment at <http://echa.europa.eu/web/guest/guidance-documents/guidance-on-biocides-legislation?panel=vol3partA>.

exposure, also secondary exposure assessment to a micro-organism should in general be qualitative or semi-quantitative because there are normally no reference values to which exposure levels could be compared.

In similarity with assessments of primary exposure, the assessment should consider the active micro-organism as well as any other toxicologically relevant compounds present in the biocidal product during the proposed conditions of use. Since micro-organisms may produce secondary metabolites upon contact with the target organism, the potential exposure to these substances, if considered toxicologically relevant (see section 4, must also be assessed.

#### **5.2.3.6 Secondary exposure to viable residues**

Ideally, realistic data on exposure levels, i.e. measured data from field studies should be used for the assessment of secondary exposure. If such data are not available, an estimate of the expected exposure level can be obtained using a calculation model. Depending on the intended use of the product, the models and input parameters commonly used in exposure assessments made for biocidal products with chemicals could be applicable also to products with micro-organisms in order to estimate the amount of product exposed to.

If measured data are not available the following information can be used to estimate secondary exposure:

- re-entry periods or other precautions to protect humans and animals;
- method of application;
- detailed information on the use area/treated surface;
- maximum application rate;
- minimum and maximum application volume;
- composition of the product;
- the persistence of the micro-organism and/or contaminants, as appropriate, on treated materials and surfaces, taking into account the influence of factors such as growth requirements temperature, UV light, pH and competition from other species etc.

#### **5.2.3.7 Secondary exposure to non-viable residues**

Micro-organisms may produce toxic metabolites, both during fermentation and when in contact with the target organism, i.e. after application of the biocidal product. This possibility must also be taken into account in the assessment of secondary exposure.

Ideally, realistic data on exposure levels, i.e. measured data from field studies should be used for the assessment of secondary exposure. If such data are not available, an estimate of the expected exposure level can be obtained using a calculation model. Non-viable residues can, in this context, be equalized with chemicals. Thus models commonly used for biocidal products with chemicals may be used in the exposure assessment.

Presently, there is an on-going activity within the OECD to develop guidance on how to assess risk from microbial metabolites. This guidance should be considered as soon as it is published. Meanwhile, the following factors could be taken into consideration when assessing the potential risk to non-viable residues:

- Biological factors:
  - The development stages/life cycle, persistence and multiplication of the micro-organism during conditions relevant for exposure should be taken

into consideration to estimate the likelihood of metabolite production inside the product and on the treated surface. The life cycle of the micro-organism, the production/formation of non-viable residues at relevant stages and the growth curves under optimal conditions could provide information on the likelihood of non-viable residues being produced at the site of application.

- The stability of the non-viable residues on the treated area, including the effects of factors such as temperature, UV light, pH and the presence of degrading enzymes or other substances that may impact on the stability.
- Exposure factors:
  - The likelihood of exposure during the intended use. In case no exposure is anticipated the risk could be expected to be insignificant. This may be the case if the product is applied only in areas that are inaccessible to humans or if the product is used in areas where humans who may come in contact with treated surfaces is protected from exposure by the normal working clothes.

## 5.2.4 Risk assessment

The aim of this section is to provide guidance on how the potential risk associated with exposure to the TGAI through the use of the biocidal product can be assessed. A more general description of the important steps in a risk characterisation is available in [Guidance on the BPR: Volume III Human Health, Part B Assessment](#).

In the context of BPR, risk means that the exposure level calculated for the intended pattern(s) of use is higher than the reference values set in the hazard assessment (see section 5.2.2 above). Generally, reference values used for risk characterisations (e.g. Acceptable Exposure Levels or AELs, Acceptable Exposure Concentrations or AECs<sup>46</sup>) are based on critical effects in toxicological studies that occur above a certain threshold. In similarity with the exposure assessment, the potential risk should be assessed taking into account each exposed population, product-type, and method of application that is relevant for the respective biocidal product. When such reference values cannot be derived, in general a qualitative or semi-quantitative assessment to a micro-organism should be acceptable. This is expected to usually be the case for micro-organisms, and therefore the exposure assessment should in general be qualitative or semi-quantitative.

### 5.2.4.1 Primary exposure

The total potential exposure to the micro-organism via inhalation, the oral or the dermal route that may arise during mixing/loading, application, cleaning or maintenance should be summarised and compared to a reference value, if available, that is relevant for the expected exposure duration (i.e. acute, short-term or long-term AELs/AECs). In case the micro-organism or the biocidal product is produced within the EU for biocidal use only, exposure during manufacture and packaging may also need to be assessed<sup>47</sup>. However, this exposure should not be included in the primary exposure assessment unless the manufacturing personnel would be the same person as the user.

If a risk has been concluded for a certain use, it may, in some situations yet be possible to reduce this risk by different measures. If the predominant exposure route is

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<sup>46</sup> Information on the calculation of AELs and AECs is provided in Volume III, Part B of the Guidance on Biocides legislation (<http://echa.europa.eu/web/guest/guidance-documents/guidance-on-biocides-legislation>).

<sup>47</sup> Guidance on the relevance of manufacturing process when carrying out the risk assessment under the BPD at <http://echa.europa.eu/guidance-documents/guidance-on-biocides-legislation/biocidal-products-directive>

inhalation, the risk may be reduced if the product is applied differently, for example, by brushing instead of spraying. The possibility to reduce primary exposure may also depend on the user category.

**Professional user:** as discussed in section 5.2.3, a professional user is expected to have access to personal protective equipment such as gloves, protective clothing and face mask including respiratory protection. Therefore, the risk can be reduced if the protection factors provided by PPE are taken into consideration in the exposure assessment.

**Non-professional user:** this user category refers to consumers. This user category is expected to use the product for a shorter duration and less frequently compared to a professional user. Non-professional users may be exposed during mixing/loading and during application. Relevant exposure paths for non-professionals need to be identified.

However, consumers are not expected to wear PPE, consequently, this type of measures cannot be considered in exposure assessment of non-professionals. Nevertheless, in the absence of appropriate test methods (see section 5.2.2) all micro-organisms are currently assumed to have the potential to cause sensitisation reactions in humans. As a consequence other types of risk mitigation measures than PPE may need to be considered where relevant. Such risk mitigation measures can be implemented, for example, at formulation, packaging or application level (e.g. restrict the use to non-powder formulations, ready-to-use packaging, easy measurement dosing, etc...).

#### 5.2.4.2 Secondary exposure including indirect exposure as a result of use

The potential risk resulting from indirect exposure of the general public after application needs to be assessed. The exposure assessment to a micro-organism will be in general be qualitative or semi-quantitative because there are normally no reference values to which exposure levels could be compared. This type of exposure which is often unintentional may be an incidental or a long-term exposure situation. Exposure may occur via dermal contact with treated areas. Hand to mouth transfer may be considered in exceptional cases. It may also result from inhalation of dust or spray mist, especially if the biocidal product is used indoors. Moreover, consumers may potentially be exposed via residues in food.

When assessing the potential risk for humans being unintentionally exposed to a micro-organism in a biocidal product, special consideration may be needed for young, old, pregnant and immune-compromised persons (YOPI), based, e.g. on exposure potential. If the number of microorganisms needs to be very high or the immune status of the individual very low in order for infection with opportunistic micro-organisms to occur, no risk for YOPI should be expected. If toxic metabolites are an issue, a risk assessment for YOPI can be performed similar to chemicals.

#### 5.2.4.3 Combined exposure

The combined exposures to the active micro-organism from all representative uses should be assessed.

### 5.3 Part C: Evaluation/conclusion/decision criteria

#### 5.3.1 Evaluation of effects on human and animal health

The decision whether or not a biocidal product containing a micro-organism is acceptable to use from a human and animal health perspective depends primarily on the toxicological/infective/pathogenic properties of the TGAI and to what extent humans and animals will be exposed to the TGAI. Acceptable means, in this context, that the micro-organism:

- lacks an ability to infect humans;
- lacks an ability to transfer genetic elements encoding resistance/cross-resistance to therapeutic human medicinal or veterinary substances;
- is rapidly cleared from the body;
- and where relevant that exposure levels are below any reference value set to protect humans and animals from any adverse effects caused by the micro-organism or any known metabolites.

The hazards of the TGAI as well as its potential to infect and cause pathogenicity depend to a great extent on the biological properties of the micro-organism, for example, its growth requirements, mode of action and ability to produce toxic metabolites, either during fermentation or after application when in contact with the target organism. Information on the production of metabolites is usually limited and data on the intrinsic toxic potential of metabolites are often even more limited. In the near future, further information on how to perform risk assessments of metabolites will be available from the OECD.

For micro-organisms the major concern is exposure via inhalation. In order to reduce the potential exposure via this route, products should be packaged in a way in which exposure of the user is minimised. Moreover, until validated test methods for skin and respiratory sensitisation have been developed, all micro-organisms should be regarded as potential sensitisers unless it can be demonstrated that there is no risk of sensitisation. The biocidal product containing the TGAI should be labelled with the phrase "Micro-organisms may have the potential to provoke sensitising reactions".

When making a decision on the authorisation of the biocidal product Member States must take into consideration all groups of the population that may be exposed, i.e. professional users, non-professional users (if relevant), and the general public exposed either directly or indirectly via the environment. This includes immunocompromised individuals. If relevant, exposure of animals should also be taken into account.

### **5.3.2 Decision criteria**

No approval will be granted if the information provided in the dossier indicates that the micro-organism is pathogenic to humans.

No approval will be granted if the information provided in the dossier clearly indicates that the micro-organism is capable of transferring genetic elements encoding resistance/cross-resistance to therapeutic human medicinal or veterinary substances.

No approval will be granted unless there is sufficient information to decide that there is no harmful effect on human or animal health arising from primary or secondary exposure to the micro-organism, its relevant metabolites/toxins and fermentation residues in the product as a result of at least one proposed use of the biocidal product, taking into consideration all identified users.

In reaching these conclusions, particular attention must be paid to vulnerable groups among the different populations.

## 6. Effects on Non-Target Organisms and Environmental Fate and Behaviour

### 6.1 Part A: Information requirements

#### 6.1.1 Effects on non-target organisms

##### 6.1.1.1 Active Micro-organism

- i. The information on identity, biological properties and further information required under sections 1 to 5 of the Annex II Title 2 of the BPR is central to the assessment of impact on the environment and non-target organisms. Additional useful information relates to fate and behaviour required under the section 9 of the Annex II Title 2 of the BPR which, together with information on the nature of the biocidal product and its manner of use, defines the nature and extent of potential exposure. The information submitted in accordance with section 5 of the Annex II Title 2 of the BPR will provide essential information as to effects to mammals and the mechanisms involved. Experimental data are normally required, unless it can be justified that an assessment of effects on non-target organisms can be performed with the information already available. It may be relevant to consider, according to the case, both micro-organisms and their spores and other resistant structures.
- ii. The choice of the appropriate non-target organisms for testing of environmental effects should be based on the identity of the micro-organism (including the host specificity, mode of action and ecology of the organism). From such knowledge it would be possible to choose the appropriate test-organisms, such as organisms closely related to the target organism. Alternative methods, such as read-across should be considered first whenever possible.
- iii. The information provided, taken together with that for one or more biocidal products containing the micro-organism, must be sufficient to permit an assessment of the impact on non-target species (flora and fauna), likely to be at risk from exposure to the micro-organism, where they are of environmental significance. Impact can result from single, prolonged, or repeated exposure and can be reversible or irreversible.
- iv. In particular, the information provided for the micro-organism, together with other relevant information, and that provided for one or more biocidal products containing it, should be sufficient to:
  - decide whether, or not, the micro-organism can be included in a Union list of approved active substances;
  - specify appropriate conditions or restrictions to be associated with any inclusion in the Union list of approved active substances;
  - permit an evaluation of risk for non-target species populations, communities, and processes as appropriate;
  - specify the precautions necessary for the protection of non-target species.
- v. All available biological data and information which is relevant to the assessment of the ecology profile of the micro-organism must be reported.
- vi. It may be necessary to conduct separate studies for relevant metabolites (especially toxins), where these products can constitute a risk to non-target organisms, and where their effects cannot be evaluated by the available results



relating to the micro-organism. Only in case of a relevant metabolite/toxin that is stable outside the micro-organism, a toxic effect of the relevant metabolite / toxin is independent of the presence of the micro-organism, and the relevant metabolite / toxin is expected to occur in the environment in concentrations higher than under natural conditions, special studies on metabolites/toxins are relevant. Before such studies are performed, the applicant must seek agreement of the competent authorities on whether such studies need to be performed.

- vii. In order to facilitate the assessment of the significance of test results obtained, the same strain (or recorded origin) of each relevant species should, where possible, be used in the various tests specified. Strains used in tests should be reported clearly.
- viii. Tests must be performed unless it can be justified that the non-target organism will not be exposed to the micro-organism. If it is justified that the micro-organism does not cause toxic effects or is not pathogenic or infective to vertebrates or plants and not closely related to species that are known pathogens, only reaction to appropriate non-target organisms must be investigated. Useful information may also be available from the observations carried out in efficacy testing.
- ix. For all studies, average achieved dose in cfu/kg body weight as well as in other appropriate units must be reported. However it may be considered whether the IU (international unit measuring biopotency) is the more relevant unit to assess the risk for the environment of the specific microorganism. This may be relevant when the mode of action of this active micro-organism is based on toxins. The biopotency is an indirect way to assess the hazard of the toxin which is not taken into account either by the mass of bacterial material nor the density (CFU) content<sup>48</sup>.



**NOTE to the reader:** The endpoints described below are numbered in accordance with the BPR, Annex II Information Requirements for Active Substances, Title 2 Micro-organisms, Core data set and additional data set for active substances.

Headings are shown in ***italic green font*** to distinguish them from the general section numbers of the Guidance document.

## ***8. Effects on non-target organisms***

Information requirements in this Section may be adapted as appropriate in accordance with the specifications of Title 1 of this Annex.

### ***8.1. Effects on aquatic organisms***

#### ***8.1.1. Effects on fish***

Information on toxicity, infectiveness and pathogenicity to fish must be reported.

#### ***8.1.2. Effects on freshwater invertebrates***

Information on toxicity, and pathogenicity to freshwater invertebrates must be reported.

#### ***8.1.3. Effects on algae growth***

Information on effects on algal growth, growth rate and capacity to recover must be reported in case the micro-organism is taxonomically related to a known plant pathogen, and is conditionally required where exposure to surface water cannot be excluded.

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<sup>48</sup> Stated by ENV Working Group regarding the *Bacillus thuringiensis kurstaki* active substance dossier.

#### **8.1.4. Effects on plants other than algae (Additional Data Set (ADS))**

Information on effects on plants other than algae must be reported in case the micro-organism is taxonomically related to a known plant pathogen.

#### **8.2. Effects on earthworms**

Potential impact on earthworms should be discussed.

#### **8.3. Effects on soil micro-organisms**

Information on impact on relevant non-target micro-organisms and on their predators (e.g. protozoa for bacterial inoculants) should be reported. Expert judgment is required to decide whether additional studies are necessary. Such decision will take into consideration the available information in this and other sections, in particular data on the specificity of the micro-organism, and the expected exposure in relation to background concentration of closely related organisms and population dynamics.

#### **8.4. Effects on birds**

Information on toxicity, infectiveness and pathogenicity to birds must be reported.

#### **8.5. Effects on bees**

Information on toxicity and pathogenicity to bees must be reported.

#### **8.6. Effects on arthropods other than bees**

Information on toxicity, and pathogenicity to arthropods other than bees must be reported. The selection of the test species should be related to the potential use of the biocidal products.

#### **8.7 Further studies (ADS)**

Additional studies may include further acute studies on additional species such as terrestrial plants, mammals or other relevant species or processes, or higher tier studies such as chronic, sub-lethal or reproductive studies on selected non-target organisms.

Before performing such studies, the applicant must seek agreement of the competent authorities on the type of study to be performed.

##### **8.7.1. Terrestrial plants (ADS)**

Covered by the guidance under the endpoint 8.7 "Further studies" above.

##### **8.7.2. Mammals (ADS)**

Covered by the guidance under the endpoint 8.7 "Further studies" above.

##### **8.7.3. Other relevant species and processes (ADS)**

Covered by the guidance under the endpoint 8.7 "Further studies" above.

#### **8.8. Summary and evaluation of effects on non-target organisms**

A summary and evaluation of all data relevant to the environmental impact should be provided. It should include a detailed and critical assessment of those data in the context of relevant evaluative and decision making criteria and guidelines, with particular reference to the risks for the environment and non-target species that may or do arise, and the extent, quality and reliability of the data base. In particular the following issues should be addressed:

- distribution and fate in the environment, limiting and favouring environmental factors and the time courses involved;
- identification of non-target species and populations at risk, and the extent of their potential exposure;

- identification of precautions necessary to avoid or minimise contamination of the environment, and for the protection of non-target species.

#### 6.1.1.2 Biocidal Product

**!** **NOTE to the reader:** The endpoints described below are numbered in accordance with the BPR, Annex III Information Requirements for Biocidal Products, Title 2 Micro-organisms, Core data set and additional data set for active substances. Headings are shown in **italic green font** to distinguish them from the general section numbers of the Guidance document.

### **9. Ecotoxicological studies**

#### **9.1. Information relating to the ecotoxicity of the biocidal product which is sufficient to enable a decision to be made concerning the classification of the product is required**

Annex III, Title 2, 9.1 Column 1 states:

- *Where there are valid data available on each of the components in the mixture and synergistic effects between any of the components are not expected, classification of the mixture can be made according to the rules laid down in Directive 1999/45/EC, Regulation (EC) No 1907/2006 (REACH) and Regulation (EC) No 1272/2008 (CLP).*
- *Where valid data on the components are not available or where synergistic effects may be expected then testing of components and/or the biocidal product itself may be necessary.*

#### **9.2 Further ecotoxicological studies**

Annex III, Title 2, 9.2 Column 1 states: *Further studies chosen from among the endpoints referred to in section 8 of Annex II "Micro-organisms" for relevant components of the biocidal product or the biocidal product itself may be required if the data on the active substance cannot give sufficient information and if there are indications of risk due to specific properties of the biocidal product.*

#### **9.3 Effects on any other specific, non-target organisms (flora and fauna) believed to be at risk (ADS)**

Such testing may be required if tests on other non-target organisms are needed on the basis of intended use(s) and results from the other tests in section 8 and 9 of Annex II Title 2 of the BPR (data set for the active substance) or a preliminary risk assessment compiled in accordance with section 12 of Annex II Title 2 of the BPR. For instance, tests on sediment dwelling organisms, aquatic plant growth (including macro-algae), accumulation and elimination in shellfish or tests on marine macro-algae or other additional tests on estuarine and marine organisms may be needed.

The decision on the need of such further studies should be decided case-by-case after consulting with the competent national authority.

Annex III, Title 2, 9.3 Column 3 states: *Data for the assessment of hazards to wild mammals are derived from the mammalian toxicological assessment.*

#### **9.4 If the biocidal product is in the form of bait or granules (ADS)**

If the biocidal product is in the form of bait or granules the studies under the endpoints 9.4.1. and 9.4.2 for biocidal products may be required.

#### **9.4.1 Supervised trials to assess risks to non-target organisms under field conditions**

If first tier data are available and indicate the micro-organism is infectious there may be a risk for secondary effects on non-target organisms. The decision on the need of such further studies should be decided case-by-case after consulting the competent national authority.

#### **9.4.2 Studies on acceptance by ingestion of the biocidal product by any non-target organisms thought to be at risk**

No guidance available.

#### **9.5 Secondary ecological effect e.g. when a large proportion of a specific habitat type is treated (ADS)**

The decision on the need of such further studies should be decided case-by-case after consulting with the competent national authority.

### **6.1.2 Environmental fate and behaviour**

#### **6.1.2.1 Active Micro-organism**

Information on the origin, the properties, and the survival of the micro-organism and its residues as well as its intended use form the basis for an assessment of environmental fate and behaviour. Experimental data are normally required unless it can be justified that an assessment of its fate and behaviour in the environment can be performed with the information already available. This justification can be based on open literature, on practical experience and on information submitted. The interactions of the micro-organism with the environment are of particular interest. It may be relevant to consider, according to the case, both micro-organisms and their spores and other resistant structures.

The information provided, taken together with other relevant information, and that for one or more biocidal products containing the micro-organism, must be sufficient to permit an assessment of its fate and behaviour as well as that of its residual traces and relevant metabolites/toxins, where they are of significance for human health and/or the environment.

In particular, the information provided should be sufficient to:

- decide whether, or not, the micro-organism can be included in a Union list of approved active substances;
- specify appropriate conditions or restrictions to be associated with any inclusion in the Union list of approved active substances;
- predict the distribution, fate, and behaviour in the environment of the micro-organism and its relevant metabolites as well as the time courses involved;
- identify measures necessary to minimize contamination of the environment and impact on non-target species.

Any relevant metabolites / toxins (*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 / toxins are present in or produced by the micro-organism, data as outlined under Annex II, section 10 for chemical active substances of the BPR, may be required, if all of the following conditions are met:

- the relevant metabolite / toxin is stable outside the micro-organism, see endpoint 3.5 for active micro-organisms (section 4.1.1 of this guidance); and

- a toxic effect of the relevant metabolite / toxin is independent of the presence of the micro-organism; and
- the relevant metabolite / toxin is expected to occur in the environment in concentrations higher than under natural conditions.

Available information on the relationship with naturally occurring wild type relatives should be taken into account.

Before performing studies as referred to below, the applicant must seek agreement of the competent authorities on whether studies need to be performed and, if so, the type of study to be conducted. The information from the other sections has, also, to be taken into account.

**⚠ NOTE to the reader:** The endpoints described below are numbered in accordance with the BPR, Annex II Information Requirements for Active Substances, Title 2 Micro-organisms, Core data set and additional data set for active substances. Headings are shown in ***italic green font*** to distinguish them from the general section numbers of the Guidance document.

## ***9. Environmental fate and behaviour***

### ***9.1. Persistence and multiplication***

Appropriate information on the persistence and multiplication of the micro-organism, in all environmental compartments has to be given, unless it can be justified that exposure of the particular environmental compartment to the micro-organism is unlikely to occur. Special attention must be given to competitiveness under the environmental conditions prevailing at and after the intended use.

Estimated levels of the specified micro-organism in a time course after use of the product under the proposed conditions of use must be given as well as information on possible dispersal routes of the micro-organism (via air as dust particles or aerosols, with host organisms as vectors, etc.). In case information on a different strain is used an explanation should be given why data can be extrapolated.

#### ***9.1.1. Soil***

Information on viability/population dynamics should be reported in cultivated and uncultivated soils representative for soils typical of the various Community regions where use exists or is anticipated, unless it can be justified that exposure is unlikely to occur. If the test organisms is to be used in association with other media than soil, this must be included in the test range.

Information on population dynamics can also be based on information on natural population dynamics and background levels in relation to exposure levels.

Information on population dynamics in soil (and water) should be reported preferably for the strain (if available), but at least for the species.

#### ***9.1.2. Water***

In case of probability of exposure of surface water, information on viability in water/sediment systems must be given. In case the organism is viable in water/sediment or can survive, information on population dynamics and the fate in water/sediment systems is required.

Information on population dynamics in water (as for soil) should be reported preferably for the strain (if available), but at least for the species.

### 9.1.3. Air

Where applicable in case of relevant exposure, or in case of concern, information should be submitted on viability and concentration of the micro-organism in the air compartment.

Information on population dynamics in air should be reported preferably for the strain (if available), but at least for the species.

### 9.1.4. Mobility

The possible spread of the micro-organism and relevant metabolites/toxins should be discussed for the relevant environmental compartments unless it can be justified that exposure of the particular environmental compartments to the micro-organism is unlikely to occur. In this context, the intended use, life cycle stages, including occurrence of vectors, persistence and the ability of the organism to colonise adjacent habitats are of particular interest.

In case of a relevant metabolite/toxin that is stable outside the micro-organism, a toxic effect of the relevant metabolite / toxin is independent of the presence of the micro-organism, and the relevant metabolite / toxin is expected to occur in the environment in concentrations higher than under natural conditions, further studies on this metabolite are relevant.

### 9.1.5. Summary and evaluation of fate and behaviour in the environment

A summary of all data and information provided under section 9 of the Annex II Title 2 of the BPR must be submitted, and include a detailed and critical assessment of those data in the context of relevant evaluative and decision making criteria and guidelines, with particular reference to the risks for the environment that may or do arise, and the extent, quality and reliability of the data base.

## 6.1.2.2 Biocidal Product



**NOTE to the reader:** The endpoints described below are numbered in accordance with the BPR, Annex III Information Requirements for Biocidal Products, Title 2 Micro-organisms, Core data set and additional data set for active substances.

Headings are shown in ***italic green font*** to distinguish them from the general section numbers of the Guidance document.

## 10. Environmental fate and behaviour

### 10.1. Foreseeable routes of entry into the environment on the basis of the use envisaged

Please follow guidance in Volume IV Part A of the *Guidance on information requirements for biocides*.

### 10.2. Further studies on fate and behaviour in the environment (ADS)

Annex III, Title 2, 10.2 Column 1 states: *Where relevant, all the information required in section 9 of Annex II "Micro-organisms" may be required for the product. For products that are used outside, with direct emission to soil, water or surfaces, the components in the product may influence the fate and behaviour (and ecotoxicity) of the active substance. Data are required unless it is scientifically justified that the fate of the components in the product is covered by the data provided for the active substance and other identified substances of concern.*

### **10.3. Leaching behaviour (ADS)**

In contrast to what is stated in Annex II Title 2 of the BPR the title should be changed to 'Mobility' as leaching behaviour is not relevant for micro-organisms.

Please follow guidance under endpoint 9.1.4 (section 7.1.2.1 of this guidance) for active micro-organisms.

### **10.4. If the biocidal product is to be sprayed outside or if potential for large scale formation of dust is given then data on overspray behaviour may be required to assess risks to bees under field conditions (ADS)**

Guidance on the BPR: *Volume IV Environment, Part A Information Requirements* may be used where relevant for micro-organisms. This guidance is available on the BPR subsection of the "support" section, on the ECHA website<sup>49</sup>.

## **6.2 Part B: Hazard, Exposure and risk assessment**

### **6.2.1 Hazard assessment**

Hazards arising from micro-organisms are not necessarily of the same nature as those presented by chemicals. Micro-organisms are unlikely to be toxic in themselves but may produce toxic metabolites during fermentation and/or in contact with the target organism. For the active micro-organism a density - effect assessment should be carried out when possible in order to quantify the density below which adverse effects in the environmental compartment of concern are not expected to occur. This density is known as ENED (expected no effect density) or PNED (predicted no-effect density) for the micro-organism. For quantification in the environment micro-organisms are quantified as population density (Teplitski, M. et al 2000).

Currently there is no guidance on how to derive the PNED as the assessment factors which are used for chemicals may not be applicable for microorganisms and some of the standard test are not applicable.. Therefore, in some cases and often for active micro-organisms, it may not be possible to establish an ENED or PNED and a qualitative or semi-quantitative estimation of the density - effect then has to be made. For any toxins/relevant metabolites present in the biocidal product a dose - response assessment should be made in a similar way as for chemicals with PNEC as a result. An EFSA literature review on environmental risk characterisation of micro-organisms used as active substance in plant protection products discusses toxins/metabolites, its fate and behaviour after production, its stability outside the micro-organism, and the resulting potential toxicity for non-target organisms (EFSA 2013). As some of the micro-organisms used in plant protection products also can be used in biocidal products the document is applicable also in the context of assessment of biocidal products containing micro-organisms under BPR in particular in relation to characteristics of metabolites/enzymes produced and in field concentration and non-target effects observed for metabolites/toxins.

### **6.2.2 Exposure assessment**

Information on the origin and properties (e.g. specificity) of the active micro-organism/its residual metabolites/toxins and its intended use forms the basis for an assessment of environmental fate and behaviour. The mode of action of the active micro-organism should also be taken into consideration. When assessing the risks of the

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<sup>49</sup> Check "Volume IV Environment" at <http://echa.europa.eu/web/guest/guidance-documents/guidance-on-biocides-legislation?panel=vol4partA#vol4>.

active micro-organism for the environment also other potentially negative capabilities have to be assessed like specific important degradation capabilities (e.g. cellulose) or synthesis (e.g. gases), if known.

The potential for persistence and multiplication of active micro-organisms has to be assessed in relevant environmental compartments unless it can be justified that particular active micro-organisms will not reach a specific compartment. Rather than using the terminology persistence, the focus should be on unwanted and desirable competitiveness.

The mobility of active micro-organisms and their residual relevant metabolites/toxins must be considered.

For each environmental compartment, an exposure assessment should be carried out when possible in order to estimate the expected density of each active micro-organism or predict the concentration of each relevant metabolite/toxin present in the biocidal product. The expected density of the active micro-organism is known as the expected environmental density (EED) whereas for the relevant metabolite/toxin, the concentration is known as the predicted environmental concentration (PEC). However, often for active micro-organisms and relevant metabolites/toxin it may not be possible to establish an EED or a PEC and a qualitative or semi- qualitative estimate of exposure has to be made.

The PEC for the toxin could be determined with the same approach as for chemicals<sup>50</sup>. Attention has to be paid to micro-organisms that can proliferate, rest or die away, *i. e.* are living organisms and therefore current exposure models for chemicals are less suitable and need to be improved.

An EED and/or a PEC, or where necessary a qualitative estimate of exposure, need only be determined for the environmental compartments to which emissions, discharges, disposal or distributions are known or are reasonably foreseeable.

The EED/PEC, or the qualitative estimation of exposure, must be determined taking account of, in particular and where appropriate:

- a) adequately measured exposure data;
- b) the form in which the product is marketed;
- c) the type of biocidal product;
- d) the application method and application rate;
- e) the biological and physico-chemical properties;
- f) likely pathways to environmental compartments and potential for adsorption/desorption and degradation, proliferation;
- g) the frequency and duration of exposure;
- h) toxins/relevant metabolites and relevant impurities present in the biocidal product.

When conducting the exposure assessment, special consideration must be given to adequately measured, representative exposure data where such data are available. Where calculation methods are used for the estimation of exposure levels, adequate models must be applied. The characteristics of these models must fulfil requirements listed in point 34 of Annex VI of the BPR.

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<sup>50</sup> See guidance *Volume IV Environment – Part B Risk Assessment* available on ECHA website at <http://echa.europa.eu/web/guest/guidance-documents/guidance-on-biocides-legislation>.



## 6.2.3 Risk assessment

### 6.2.3.1 Active Micro-organism

The risk for the environment should be assessed where the possibility of the exposure of organisms has been established under the proposed conditions of use; if this possibility exists they must evaluate the risks arising. The potential of an active micro-organism to establish itself in the environment and therefore having a long-lasting or permanent impact on microbial communities or their predators should be considered. The transport, short-range and long-range, of the active micro-organism in the atmosphere should be taken into account.

This assessment will take into consideration the following information:

- a) the biological properties of the active micro-organism;
- b) the survival of the active micro-organism in the environment;
- c) conditions for proliferation;
- d) its ecological niche;
- e) the natural background level of the active micro-organism, where it is indigenous;
- f) information on fate and behaviour in the various parts of the environment.

Where applicable, an assessment of infectivity/infectiveness and pathogenicity is necessary, unless it can be justified that non-target organisms will not be exposed.

To assess the possibility of exposure the following information should also be taken into consideration:

- a) the survival of the active micro-organism in the respective compartment;
- b) where relevant, other authorised uses of the biocidal product in the area of envisaged use containing the same active micro-organism or which give rise to the same residues.

An active micro-organism may give rise to risks because of its potential to infect and multiply in host systems. Whether or not identified risks could be changed due to the formulation of the biocidal product must be assessed, taking into account the following information on the micro-organism:

- a) its mode of action;
- b) other biological properties;
- c) information on toxicity, pathogenicity and infectivity/infectiveness.

For micro-organisms there are few models for performing risk assessment, for further guidance see OECD working document on assessment of environmental risk (OECD Series on Pesticides No 67). The nominal level of micro-organisms can be estimated with the knowledge of the product and the application method, assuming the formulation has no influence on the dissemination of active micro-organisms. An approximate risk assessment can be performed.

For any given environmental compartment, the risk characterisation must, whenever possible, entail comparison of the EED with the ENED or PNED so that an EED/ENED or EED/PNED ratio may be derived. For active micro-organisms it may not be possible to derive the EED/ENED ratio, then the risk characterisation must entail a qualitative evaluation of the likelihood that an effect is occurring under the current conditions of exposure or will occur under the expected conditions of exposure.

Quantitative risk assessment has been performed for biocidal use as a larvicide. The model and the calculations are further described in an OECD report (OECD Series on Pesticides No 64).

A biocidal product may give rise to toxic effects due to the action of metabolites/toxins or co-formulants. For the assessment of such effects, the following information should be taken into consideration:

- a) studies on toxicity;
- b) information on fate and behaviour in the various parts of the environment.

If mortality or signs of intoxication are observed in the tests the assessment must include a calculation of toxicity/exposure ratios based on the quotient of the effect value and the estimated exposure.

#### **6.2.3.2 Biocidal Product**

The risk assessment must take account of any adverse effects arising in any of the three environmental compartments - air, soil and water (including sediment) - and of the biota, following the use of the biocidal product. The exposure pattern, the biocomplexity of the ecosystems and interactions in the microbial communities concerned must be taken into account.

#### **6.2.3.3 Risk assessments for products containing more than one Active Micro-organism**

No guidance is available yet specifically for the evaluation of several micro-organisms in one biocidal product. The general principles described in this guidance nevertheless apply.

#### **6.2.3.4 Risk assessment for micro-organisms of concern and substances of concern**

Micro-organisms of concern are known human pathogens and should not be included in microbial biocidal products.

For definition of "substances of concern" refer to the Article 3(f) of the BPR.

The general principles described in this guidance nevertheless apply.

### **6.3 PART C Evaluation/conclusion/decision criteria**

#### **6.3.1 Active Micro-organism**

##### **6.3.1.1 Evaluation of environmental fate/ecotox data**

Sufficient information is necessary on the possible persistence/competitiveness of the micro-organism and relevant secondary metabolites/toxins in or on materials, foodstuffs or feedstuffs under the environmental conditions prevailing at and following the intended use.

Member States must ensure that the available information is sufficient to permit a decision to be taken as to whether or not there may be unacceptable effects on non-target species (flora and fauna), due to exposure to the biocidal product containing the micro-organism following its intended use.

### 6.3.1.2 Evaluation of the risk assessment for the Active Micro-organism

Member States must evaluate the risk for the environment where the possibility of the exposure of organisms has been established under the proposed conditions of use; if this possibility exists they must evaluate the risks arising. An active micro-organism may give rise to risks because of its potential through multiplication to establish itself in the environment and can therefore have a long-lasting or permanent impact on microbial communities or their predators. The transport, short-range and long-range, of the active micro-organism in the atmosphere should be taken into account.

This evaluation will take into consideration the result of the risk assessment as described at 6.2.3.1.

Member States must evaluate the possibility of exposure of non-target organisms under the proposed conditions of use and if this possibility exists they must evaluate the risks arising for the non-target organisms concerned.

### 6.3.1.3 Decision criteria

No authorisation should be granted unless there is sufficient information to conclude that there are no harmful effects on organisms in any environmental compartment due to exposure to micro-organisms, their relevant metabolites/toxins and fermentation residues in the product as a result of at least one proposed use of the biocidal product. All identified users should be taken into account.

No authorisation should be granted if contamination of ground water or drinking water is expected as a result of the use of a biocidal product under the proposed conditions of use and the contamination may lead to unacceptable risks.

No authorisation should be granted if it is known that transfer of genetic material from the micro-organism to other organisms, may lead to unacceptable effects on the environment.

## 6.3.2 Biocidal Product

### 6.3.2.1 Evaluation of the risk assessment for the Biocidal Product

In rare cases the formulation can result in other risks than with the active micro-organism and those have to be considered. Further considerations are required if the EED/ENED or EED/PNED for the active micro-organism or PEC/PNEC is > 1. Refinements in the assessment should be a first step.

### 6.3.2.2 Evaluation of the PBT/vPvB assessment (= > exclusion/substitution criteria) – only formulation

The PBT/vPvB assessment does not concern micro-organisms. However, in case the formulation should include PBT/vPvB substances these need to be assessed. In case relevant metabolites are present in relevant amounts in the product that are PBT/vPvB substances, these need to be assessed.

Guidance on how to conduct a PBT assessment as well as the screening criteria and information for the identification of PBT/vPvB properties on substances can be found in the "*Guidance on information requirements and chemical safety assessment Chapter R.11: PBT Assessment*" according to the criteria stated in Annex XIII of the Regulation (EC) No 1907/2006 (REACH). Nevertheless, the information requirements for the BPR may be different from those under REACH.

#### 6.3.2.3 **Decision criteria**

No authorisation should be granted in case the formulation should contain PBT/vPvB substances.

## 7. References

### EU Commission documents

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TNsG on Product Evaluation – Chapter 6.2 (33rd CA meeting May 2009). Revision of Chapter 6.2 (Common Principles and Practical Procedures for the Authorisation and Registration of Products) of the TNsG on Product Evaluation and a revision of Chapter 101 (Assessment for the potential for resistance to the active substance) of the TNsG on Annex I Inclusion. (endorsed at the 33rd meeting of representatives of Members States Competent Authorities for the implementation of Directive 98/8/EC concerning the placing of biocidal products on the market (13-15 May 2009).  
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SANCO/10754/2005 rev.5 15 April 2005: Guideline developed within the Standing Committee on the Food Chain and Animal Health on the taxonomic level of micro-organisms to be included in Annex I to Directive 91/414/EEC  
[http://ec.europa.eu/food/plant/protection/resources/taxonomic\\_level\\_dir91414.pdf](http://ec.europa.eu/food/plant/protection/resources/taxonomic_level_dir91414.pdf).

Guidance Document on the assessment of new isolates of baculovirus species already included in Annex I of Council Directive 91/414/EEC (2013-05-28)  
[http://ec.europa.eu/food/plant/protection/resources/guidance\\_document\\_baculovirus.pdf](http://ec.europa.eu/food/plant/protection/resources/guidance_document_baculovirus.pdf).

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## EFSA Guidance

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<http://www.efsa.europa.eu/en/supporting/pub/518e.htm>.

## OECD Guidelines

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[http://search.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/jm/mono\(2005\)14&doclanguage=en](http://search.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/jm/mono(2005)14&doclanguage=en).

OECD Series on Harmonisation of Regulatory Oversight in Biotechnology No 20: Consensus Document on Information Used in the Assessment of Environmental Applications Involving Baculoviruses (2013-05-28)

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