Risk assessment for microbial pesticides

Summary report from the tasks performed by the Dutch working group on microbial pesticides

Gertie H.P. Arts

WAGENINGEN
UNIVERSITY & RESEARCH

Risk assessment for microbial pesticides

Summary report from the tasks performed by the Dutch working group on microbial pesticides

Gertie H.P. Arts

This research was subsidised by the Dutch Ministry of Agriculture, Nature and Food Quality (project number BO-43- 102.01-010).

Wageningen Environmental Research
Wageningen, June 2023

Reviewed by:
Dr. Louise Wipfler, BO programme coordinator, team Environmental Risk Assessment, Wageningen Environmental Research

Approved for publication:
Ms. S. Ahrari, Teamleader of team Environmental Risk Assessment

Report 3267
ISSN 1566-7197
ISBN 978-94-6447-750-4

WAGENINGEN
UNIVERSITY & RESEARCH

Arts, G.H.P., 2023. *Risk assessment for microbial pesticides; Summary report from the tasks performed by the Dutch working group on microbial pesticides.* Wageningen, Wageningen Environmental Research, Report 3267. 24 pp.; 0 fig.; 0 tab.; 16 ref.

In 2019 a Dutch scientific working group on microbial pesticides was established. Within this working group a number of organisations are represented (Wageningen Environmental Research (chair), Dutch Board for the admission of pesticides (Ctgb), Wageningen Plant Research, National Health Institute in The Netherlands (RIVM); Netherlands Institute of Ecology (NIOO), and University of Utrecht). The aim of this scientific working group is to work on proposals for a scientifically underpinned simplification of the risk assessment for microbial pesticides. These proposals are used by the Dutch Board for the admission of pesticides (Ctgb), as a contribution to the European working group on microbial pesticides in which a number of member states are represented. This report presents an overview of the topics discussed in the Dutch working group.

Keywords: microbial pesticides, risk assessment, antimicrobial resistance, secondary metabolites, fate, indirect effects

This report was collated for the ministry of LNV.

The pdf file is free of charge and can be downloaded at https://doi.org/10.18174/633070 or via the website www.wur.nl/environmental-research (scroll down to Publications – Wageningen Environmental Research reports). Wageningen Environmental Research does not deliver printed versions of the Wageningen Environmental Research reports.

Wageningen Environmental Research Report 3267 | ISSN 1566-7197

Photo cover: *Bacillus amyloliquefaciens* Science Trends

Contents

Verification **5**

Preface **7**

Summary **9**

1 Introduction **11**

2 Antimicrobial resistance **12**

2.1 Antimicrobial compounds 12
2.2 Antimicrobial resistance 12
2.3 New Guidelines recently approved 13

3 Secondary metabolites **14**

4 Endophytes and epiphytes **16**

4.1 Research question 16
4.2 Outcome of the literature search 16

5 Environmental fate and behavior **17**

6 Evaluation of effects on non-target microorganisms **18**

7 Decision tree IBMA (International Biocontrol Manufacturers Association) **20**

8 Adapted uniform principles and data requirements and other topics **21**

References **23**

Verification

Report: 3267
Project number: BO-43- 102.01-010

Wageningen Environmental Research (WENR) values the quality of our end products greatly. A review of the reports on scientific quality by a reviewer is a standard part of our quality policy.

Approved reviewer who stated the appraisal, and approved for publication,

position: BO programme coordinator

name: Dr. Louise Wipfler, team Environmental Risk Assessment, Wageningen Environmental Research

date: 21 March 2022

Approved team leader responsible for the contents,

name: Ms. S. Ahrari

date: 31 May 2023

Preface

In 2019 a Dutch scientific working group on microbial pesticides was established. Wageningen Environmental Research (Gertie Arts) is chairing this working group. Within this working group a number of organisations are represented (National Health Institute in The Netherlands; Wageningen Plant Research, Wageningen Environmental Research, Netherlands Institute of Ecology (NIOO), Dutch Board for the admission of pesticides (Ctgb) and University of Utrecht (UU). The aim of this scientific working group is to work on proposals for a scientifically underpinned simplification of the risk assessment for microbial pesticides. These proposals are used by the Dutch Board for the admission of pesticides (Ctgb), as a contribution to the European working group on microbial pesticides in which a number of member states are represented.

Working group members

In the Dutch working group on microbial pesticides held the following members:

Gertie Arts (WENR, voorzitter)
Mechteld ter Horst (WENR)
Rob van Drent (Ctgb) (2018 – 2020)
Jacobijn van Etten (Ctgb)
Anne Steenbergh (Ctgb)
Jacqueline Scheepmaker (RIVM) (2018 – 2020)
Rob de Jonge (RIVM)
Boet Glandorf (RIVM)
Peter Bakker (Universiteit Utrecht)
Jürgen Köhl (WPR)
Willem Jan de Kogel (WPR)
Wietse de Boer (NIOO)

Summary

The Ministry of Agriculture, Nature and Food Quality in the Netherlands has requested Wageningen Research to elaborate on a scientifically justified simplification of the risk assessment for microbial pesticides and their approval, involving all expertise deemed necessary and to provide supportive scientific input for Ctgb as NL representative in the European working group on biopesticides. This report presents an overview of the topics discussed in the Dutch scientific working group since its establishment in June 2019. Wageningen Environmental Research leads this process in the Dutch Working Group and is chair. The other members of the Dutch working group represent the other organisations (NIOO, WUR WPR, RIVM, UU, Ctgb). The Dutch working group has a multidisciplinary composition, all contributing to the risk assessment of micro-organisms.

Between 2019 and August 2021 topics were discussed that were relevant to the risk assessment of microbial pesticides used as crop protection products. The topics that were discussed specifically related include antimicrobial compounds and antimicrobial resistance; secondary metabolites; endophytic versus epiphytic lifestyle; environmental fate and behaviour; effects on non-target micro-organisms; other topics related to the data requirements and uniform principles.

The Dutch scientific working group (Gertie Arts (WENR, chair), Rob van Drent (Ctgb), Jacobijn van Etten (Ctgb), Anne Steenbergh (Ctgb), Jacqueline Scheepmaker (RIVM), Rob de Jonge (RIVM), Boet Glandorf (RIVM), Peter Bakker (Universiteit Utrecht), Jürgen Köhl (WPR), Willem Jan de Kogel (WPR), Wietse de Boer (NIOO), Mechteld ter Horst (WENR), Cláudia de Lima e Silva (WENR)) discussed the topic of antimicrobial resistance. The risk assessment should assess if this resistance or its possible transfer via resistance genes does interfere with the effectiveness of highly or critically important antimicrobials used in human and animal health and lead to adverse effects on humans and animals. The Ctgb co-authored the guidance document on antimicrobial resistance which was developed by the Biopesticide working group and endorsed by the Standing Committee on Plants, Animals, Food and Feed in October 2020.

The discussion about secondary metabolites has already been conducted for a very long time. What confused the discussion is that until recently the data requirements for secondary metabolites of micro-organisms were based on degradation products/metabolites of chemicals. For biocontrol micro-organisms, the concern is the potential production of toxic substances and how to evaluate the associated risk. From a scientific review it was advised to implement a basic hazard assessment. If this hazard assessment is passed without any concerns, the production of unknown, potentially toxic, substances does not need to be further investigated. Ctgb co-authored the EU Guidance document based on this approach on which consensus was reached by the EU Working Group on Biopesticides. The guidance document was endorsed by the Standing Committee on Plants, Animals, Food and Feed in October 2020.

The question if a biocontrol micro-organism lives endophytically (=living inside a plant) or epiphytically (= living on the outer parts of the plant) is of concern as during its endophytic growth, these organisms could produce high concentrations of metabolites resulting in negative effects on human health, e.g. when the plants are crops and consumed by humans. Many micro-organisms are optionally endophytic. So, according to the Dutch scientific working group, ruling out endophytic growth based on the identity of the biocontrol micro-organism is not a good criterium to be applied in any guidance. Therefore, the NL working group came to the conclusion that this criterium is not adequate given the transient presence of endophytic biocontrol microorganisms and the overall low concentrations in which they occur. This conclusion was underpinned by a literature research (Scheepmaker, 2021).

The Dutch scientific working group supported the Ctgb proposal to require an assessment of the hazard first and the assessment of behaviour as a next step. This proposal was subsequently taken up by the Biopesticides working group. The rationale was that data on fate and behaviour of micro-organisms used for biocontrol might only be needed if toxicity tests show effects and thus one or more of the environmental or

human protection goals is threatened. An extra consideration was that measuring environmental fate and behaviour of microbial biocontrol organisms is very difficult, as it requires data about population densities and background levels. In the NL working group it was raised that biocontrol intends to introduce high population densities into the cropping system. These densities decrease over time due to environmental factors. This is underpinned by many research projects and published papers. The Ctgb drafted a revision of the fate and behaviour chapter of the data requirements and took into account the input of the NL working group. The proposed view was the approach that in case hazards are identified for humans or the environment, data on the population dynamics of the microorganism upon application may be used in the risk assessment. The NL working group has commented on the fate and behaviour paragraph via the public consultation in September 2021.

The Dutch working group on biopesticides discussed the need for any data requirements concerning the effect of a biocontrol micro-organism on non-target micro-organisms. The background of this question was that the general principles for the revision of the data-requirements should be to ask for strictly necessary information only. Until recently, data-requirements include the evaluation of effects on non-target micro-organisms. The group evaluated a position paper by Sundh (2020) and agreed with the statement of the author, where he concludes that available scientific evidence shows that it is unlikely that use of biocontrol micro-organisms will have major effects on non-target microbes since populations of the biocontrol micro-organisms decline to background levels over time. In the position paper, several considerations were raised that are in favour of skipping the data-requirements for an evaluation of effects on non-target soil micro-organisms. The group supports this position paper and has added additional arguments to it. The Ctgb successfully advocated for the removal of this data requirement for most microorganisms, however effects on soil microbial function should still be evaluated and submitted in the dossier.

A number of other topics was discussed. One of those was if the fact whether a micro-organism is indigenous or not is relevant to the risk assessment. The working group noticed a couple of difficulties with assessing the criterium of indigenousness if this was a data requirement. Firstly, it is unclear what the relevant geographical scale is to assess whether a microorganism is indigenous (e.g., in each country separately or for the EU as a whole). Secondly, the taxonomical level at which the micro-organism would be indigenous or not is unclear (e.g., at species-level or at strain-level). In the revised data requirements (adopted since 21 November 2022) the term indigenous is avoided, while information on the geographical origin of the strain and the natural occurrence of the species can be used to inform the risk assessment.

The Dutch scientific working group discussed the topic of invasiveness. A microorganism is considered to be invasive when it is capable of spreading and growing in new ecosystems and exerts negative influence on local populations or ecosystems. From the scientific literature, there is little evidence for the occurrence of non-pathogenic, invasive microorganisms and many papers support the view that populations of micro-organisms used as microbial pesticides generally decline upon application and will not become invasive. This scientific insight was conveyed to the EU WG biopesticides. This WG did not adopt the topic of invasiveness as a separate criterium in the data requirements.

The International Biocontrol Manufacturers Association (IBMA) proposed a decision tree, that was discussed in the NL working group. This decision tree is an attempt to discriminate among several groups of micro-organisms at an early stage in the risk assessment. The NL working evaluated the decision tree as an interesting approach and suggested some improvements to the tree.

The new uniform principles and the new data requirements were accepted by the European Commission and came into force on 21 November 2022. For reference see:
https://food.ec.europa.eu/plants/pesticides/micro-organisms_en

1 Introduction

This report focuses on microbial biological control agents (MBCAs; the term Microbial Active Substance is used in the data requirements), which are agents that contain micro-organisms (and their metabolites) as their 'active substances' instead of chemicals. These MBCAs take advantage of fundamental ecological interactions between organisms. MBCAs include bacteria, fungi, yeasts, viruses, protozoa and microsporidia a.o. which target organisms including insects, mites, nematodes, weeds and other micro-organisms such as fungi or bacteria causing plant diseases. MBCAs are often host-specific as specific organisms are needed for each pathogen and crop. Biocontrol agents are used in Integrated Pest management (IPM) to either reduce or replace chemical pesticides. Current political ambitions focus on the implementation of IPM at the level of Europe and in The Netherlands. This implies that there is a request for alternative pest and disease control agents.

Microbial pest control agents (MBCAs) are promising for use in sustainable agriculture. Nevertheless, they face a major hurdle in the registration process of the European Union, one reason being unclear data requirements, which were originally based on chemical compounds. At the European level a working group including a number of Member States (around 12) has worked on proposals for the revision of the data requirements and the uniform principles for microbial pesticides.

This report presents an overview of the topics discussed in the Dutch scientific working group since its establishment in June 2019. The new uniform principles and the new data requirements were finalized on 21 November 2022. For reference: https://food.ec.europa.eu/plants/pesticides/micro-organisms_en. The new data requirements for micro-organisms used as active ingredient in microbial pesticides include a separate part (part B, active substances that are micro-organisms) that is dedicated to these type pf pesticides. This is the part that has been adapted and made more fit-for purpose for microbial pesticides.

2 Antimicrobial resistance

2.1 Antimicrobial compounds

The presence of detectable amounts of medically important antimicrobial compounds in the formulated product is considered to pose an unacceptable risk to human and animal health, unless it is demonstrated that the expected concentrations in environmental compartments are below the threshold for the formation of resistance under realistic conditions of use (EC, 2020). This refers to all antimicrobial agents important for therapeutic use in humans as described in the WHO list (WHO, 2019) of Critically important Antimicrobials (CIA), Highly Important Antimicrobials (HIA) and Important Antimicrobials (IA). (EC, 2020).

Many organisms – but not all - produce antimicrobial compounds. If the antimicrobial compounds are present in the product in detectable amounts, the WHO list needs to be applied and taken into account in the risk assessment. If the detected compound is present on the WHO list, further assessment is needed.

2.2 Antimicrobial resistance

The European Commission (EC, 2020) has recently published a guideline about antimicrobial resistance. This emerges when micro-organisms (such as bacteria and fungi) adapt in response to exposure to antimicrobial drugs (EC, 2020). Actually, antimicrobial resistance is an adaptive trait that enables microbial subpopulations to survive and overcome host strategies aimed against them. Some micro-organisms are intrinsically resistant to certain types of antimicrobials. Bacteria, in particular, may acquire antimicrobial resistance via mutation(s) in chromosomal genes and/or via acquisition of antimicrobial resistance genes from other bacteria of the same or of different species. As a result, the microorganism can become resistant to an antimicrobial to which it was previously susceptible (EC, 2020).

The WHO (2015) regards emergence of antimicrobial resistance in human-pathogenic micro-organisms as one of the biggest threats to human health, according to the published report: "Global action plan on antimicrobial resistance" (WHO, 2015).

The European Commission (EC, 2020) mentions a series of knowledge gaps that exist. These include the question how the use of microbial pest control agents and products (MBCA/MPCP) could affect micro-organisms that are present in the ecosystem in regard to the triggering of specific antimicrobial mechanisms. The potential long-term consequences of releasing MBCAs potentially containing transferable antimicrobial resistance genes into the environment must thus be assessed. Applying micro-organisms in the environment by spreading them as plant protection products may potentially contribute to antimicrobial resistance, through the spread of genes which can be horizontally transmitted from the microbial pest control agent to pathogenic bacteria (EC, 2020).

Currently the only low-risk criterion for micro-organisms considers "multiple antimicrobial resistance" (EC, 2020). The text reads as follows: "An active substance which is a microorganism may be considered as being of low-risk unless at strain level it has demonstrated multiple resistance to antimicrobials used in human or animal medicine" (EC, 2020).

The Uniform Principles outline that it should be demonstrated that, if the micro-organisms are resistant to antimicrobial(s), this resistance or its possible transfer does not interfere with the effectiveness of antimicrobials used in human and animal health care or that this possible transfer does not lead to adverse effects on human and animal health. When the resistance can be transferred to other micro-organisms, including human and animal pathogens, the microorganism should not be approved.

Whole genome sequencing can be used to identify antimicrobial resistance genes. When the search for antimicrobial resistance genes is required, it is recommended to conduct it against at least two maintained databases.

The Dutch scientific working group discussed the topic of antimicrobial resistance. The risk assessment should address issues if this resistance or its possible transfer via resistance genes does interfere with the effectiveness of highly or critically important microbials used in human and animal health and lead to adverse effects on humans and animals. The Ctgb co-authored the guidance document on antimicrobial resistance which was endorsed by the Standing Committee on Plants, Animals, Food and Feed in October 2020.

The new data requirements have defined "metabolite of concern" meaning a metabolite produced by the micro- organism under assessment, with known toxicity or known relevant antimicrobial activity, which is present in the microbial pesticide product as manufactured at levels that may present a risk to human health, animal health or the environment, and/or for which it cannot be adequately justified that in-situ production of the metabolite is not relevant for the risk assessment.

2.3 New Guidelines recently approved

The guidance on the approval of low-risk criteria linked to antimicrobial resistance for microbial active substances was recently approved (see link below). The Ctgb co-authored the guidance document, together with Sweden and Germany.

Guidance document on the approval of low-risk criteria linked to antimicrobial resistance for microbial active substances.

3 Secondary metabolites

Microorganisms are known to produce metabolites. Metabolites are components produced by microorganisms, e.g. bacterial toxins or mycotoxins. Some of them may have toxicological significance, and some of them may be involved in the mode of action of the plant protection product. They are very different from chemical metabolites, the latter being breakdown products of plant protection products (Scheepmaker et al., 2019). Primary metabolites are directly involved in general metabolism required for basic life processes such as growth, development and reproduction of a microorganism and are typically key components in maintaining normal physiological processes (EC, 2020, Scheepmaker et al., 2019). Therefore, primary metabolites are not metabolites of potential concern and are out of the scope for the risk assessment (Scheepmaker et al., 2019). Secondary metabolites are not essential for the primary metabolic processes of microorganisms and show several biological activities possibly related to survival functions of the microorganism, such as competition, parasitism or symbiosis and metal transport. In the risk assessment, metabolites should therefore be understood as secondary metabolites (Scheepmaker et al., 2019).

Metabolites are normally produced by the microorganism under specific physical and biological conditions. The capacity of an individual strain to produce metabolites of concern depends on many environmental and genetic parameters specific for this strain. The absence of production of undesirable compounds for one or more strains therefore can not always be extrapolated to all other strains within the same species.

Some secondary metabolites may simply be a by-product of fermentation and are not involved in the mode of action of the MBCA (OECD, 2018). Several procedures exist to remove these from the medium to keep the amount of secondary metabolites in the product as low as possible. Even if the secondary metabolite is not involved in the mode of action, it is important that a risk assessment is conducted (OECD, 2018).

The topic of secondary metabolites has already been discussed for a long time and was also repeatedly a topic in the NL scientific working group.

Scientific research has been performed to shed more light on metabolites produced by biocontrol micro-organisms and when they need to be assessed in the risk assessment. Scheepmaker et al. (2019) have collated a review examining the EU regulatory perspective on metabolites of MBCAs for plant protection and identified some key issues and concerns. The previous data requirements for secondary metabolites of micro-organisms are based on degradation products/metabolites of chemicals. They conclude that this has strongly contributed to the current confusion regarding how to best evaluate potential production of toxic substances by MBCAs. They suggest that data requirements should be revised and/or guidance set-up that is fit for purpose, in order to give the EU-regulation for MBCAs a stronger base in microbiological knowledge. They also suggest implementation of a hazard-based risk assessment. If there is no hazard identified, the production of unknown, potentially toxic, substances does not need to be further investigated.

Together with Sweden, the Ctgb co-authored the EU Guidance document based on this hazard-based approach (but modified) on which consensus was reached in the EU Working Group on Biopesticides. It is described in a new guidance document (EC, 2020) that is based on the consensus reached by the EU Working Group on Biopesticides and endorsed by the Standing Committee on Plants, Animals, Food and Feed.

The guidance document has been published on-line:

Guidance on the risk assessment of metabolites produced by microbial active substances

In this guidance document (EC, 2020), the approach to assess all metabolites produced by a microorganism through an evaluation as performed for chemical active substances is not followed; it is considered as not

feasible and is deemed unnecessary from a risk perspective. However, the guidance states that parts of such an assessment are needed under certain circumstances. Such an approach ensures that applicants provide all available data on metabolites including any indication of hazardous effects of any of these metabolites and that a hazard-based approach is followed. For those metabolites for which a hazard is identified, this identified hazard needs a follow-up by generating additional data to support a further risk assessment for those particular metabolites (EC, 2020).

The data requirements are focused on identification of metabolites of (potential) concern. These apply to both metabolites in the products and those produced *in situ*, unless otherwise indicated. To determine whether the microorganism is producing a metabolite of concern, the guidance document follows a "step-by-step" procedure. Please note that both data requirements and uniform principles are currently under review. At the moment the present guidance document was accepted by the Standing Committee on Pesticides. The Netherlands (Ctgb) has asked to evaluate this guidance document for its effectivity and proportionality based on experience obtained with a number of dossiers. This request was supported by several member states.

4 Endophytes and epiphytes

4.1 Research question

Questions were posed by National Authorities if current data requirements may need to be adapted for MBCAs that can live endophytically in plants (= live inside the plant). Questions are based on the concern that during its endophytic growth, MBCAs could produce higher concentrations of metabolites resulting in negative effects on human health e.g. when the plants are crops and consumed by humans. If an endophytic lifestyle of a microorganism poses a higher risk, it would be helpful for the risk assessment to demonstrate that endophytic growth of MBCAs can be excluded.

A preliminary literature search was performed to investigate whether endophytes need further attention in the risk assessment for humans and the environment (Scheepmaker, 2021). This question as well as the results were discussed in the Dutch working group on microbial agents.

4.2 Outcome of the literature search

Scheepmaker (2021) indicates that MBCAs are able to live both epiphytically as well as endophytically and that it is not possible to exclude endophytic growth during the lifespan of the MBCA in or on the crop. This also does not change by using a different method of MBCA application on crops.

However, based on the limited survey by Scheepmaker (2021), she concluded that there are no reasons to adjust the current data requirements based on the potential of an MBCA to live endophytically. First of all the available literature indicates that MBCAs are only transiently present in plants as endophytes. Moreover, they are detected at low concentrations in the plant. Secondly, there are no indications that MBCAs produce metabolites, or concentrations of metabolites that are harmful for human health in case they grow endophytically.

The final conclusion of Scheepmaker (2021) is that, based on this literature search, the current framework is also adequate to assess potential risks of MBCAs when they grow endophytically.

The suggested adaption to the guidance is that it needs to be described where the organism lives and what its lifestyle is. Many micro-organisms are optionally endophytic. So, according to the Dutch scientific working group, absence of endophytic growth is not a good criterium to be applied in any guidance. In the proposed decision tree of IBMA (International Biocontrol Manufacturers Association), an endophytic lifestyle of biocontrol microorganism has been used as a criterium. The NL working group does not consider this criterium adequate given the transient presence and low concentrations of endophytic biocontrol microorganisms in crops. In line with the position of the NL working group, no additional criteria were included in the revised data requirements based on the capacity of a microorganism to have an endophytic life cycle.

5 Environmental fate and behavior

The NL working group discussed the proposal of the Ctgb for a simplification of the data requirements for fate and behavior. The opinion of the working group members was that data on fate and behavior of MBCAs might only be needed if toxicity tests show effects and thus one of the protection goals is threatened. It was discussed that data for fate and behavior are not always needed and, moreover, difficult to apply.

The NL working group identified some problems with the data requirements with respect to fate and behavior as explained above:

1. What is the relevant taxonomic level (strain, species ?) for which fate and behavior data is required;
2. What are the environmental conditions for which these data should be provided? (one 'representative' condition versus rather endless combinations of environmental conditions including extreme situations);
3. How can you measure the population density of a multicellular microorganism such as a fungus ?
4. The currently used concept of a natural background level as a reference is not useful given the large fluctuations in community composition.

In the working group it was raised that biocontrol intends to introduce high population densities into the cropping system. These densities decrease over time due to environmental factors. This is underpinned by many research projects and published papers (see also chapter 6).

The Ctgb proposal in the EU biopesticide working group requires an assessment of the hazard first and the assessment of behavior as a next step. The NL working group supported the Ctgb proposal, which was subsequently taken up by the Biopesticides working group.

The NL working group commented on the fate and behavior paragraph via the public consultation in September. The description of the background level of a metabolite is still required in the new data requirements.

6 Evaluation of effects on non-target microorganisms

The Dutch working group on biopesticides discussed the need for any data requirements concerning the effect of a biocontrol micro-organism on non-target micro-organisms. The background of this question was that the general principles for the revision of the data-requirements should be to ask for strictly necessary information only. The current data-requirements include the evaluation of effects on non-target micro-organisms.

The Ctgb presented a draft proposal to the NL working group based on a position paper of Sundh (2020), one of the EU WG biopesticides independent experts. In that proposal, a number of considerations were raised that are in favor of skipping the data-requirements of an evaluation of effects on non-target soil micro-organisms (see Sundh, 2020):

- Soil microbes and microbes associated to crops are extremely important for sustainable agriculture. From this point of view, micro-organisms used in plant protection can be seen as members of this soil microbes community and the plant microbiome.
- Populations of introduced microorganisms always decline due to the natural biological buffering of the environment to levels that are within common fluctuations and ranges without strongly affecting microbial communities (Köhl et al., 2019). Effects are transient and this is supported by many papers (e.g. Cordier et al., 2009).
- Microbial community structure is highly dynamic; environmental fluctuations and agricultural practice (e.g., ploughing, use of manure and compost) will constantly change the community structure.
- In case of non-indigenous microorganisms, the data requirements and uniform principles already ask information for pathogenic organisms and their effects on non-target organisms (including) non-target soil microorganisms. For non-pathogenic microorganism no additional information is considered to be needed.
- Micro-organisms which are not known to occur in EU agricultural cropping systems at species level can be invasive if the organisms are pathogenic. In case of micro-organisms pathogenic to bacteria and fungi, the risk to fungi in agricultural cropping systems should be addressed (e.g., by providing information on the host range, or by providing experimental data on the effects of the use of the microorganism on specific microbial processes).

The Dutch working group evaluated the position paper by Sundh (2020) and the Ctgb draft proposal. The Dutch Working group on Microbial Pesticides agreed with the statement of Sundh (2020). The group supports the science and also the proposed risk assessment. The Dutch Working group on Microbial Pesticides has added additional arguments to this position paper.

Sundh (2020) stresses that we have to do with rather heavily managed agroecosystems, in which a microbial balance is questionable. He explains and underpins the points listed above. EFSA supported a systematic review of environmental impacts of MBCAs which was published in 2013 (https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/sp.efsa.2013.EN-518). However, this review focuses more on the 'fate' and 'background population' of the MBCA and notes a general shortage of studies of effects of MBCAs on microbial communities, also referring to similar conclusions by Scheepmaker & Kassteele (2011). The main conclusion of the EFSA review was that available evidence suggests that it is unlikely that MBCA use will have major effects on non-target microbes since populations of the MBCA decline to background levels over time. The report recommends more studies on the biogeography and indigenousness of MBCAs.

The OECD guidance to the environmental safety evaluation of microbial biocontrol agents (Series on Pesticides No. 67, 2012) discusses the reasons that in the USA data on effects on microbial populations are not required. The guidance claims that impacts on microbial community structure or on AM fungi may be relevant in special cases, but no examples are given and there are no recommended tests. It also refers to the major conclusion of the Scheepmaker and Kassteele (2011) study, that initial effects on numbers of non-

target soil microorganisms can be both positive and negative, and recovery usually takes place within 100 days.

In summary, Sundh (2020) concludes:

1. Expected impacts can be positive or negative but recovery is usually within 100 days.
2. There is a challenge translating a (often subtle) change in community structure to a sound and understandable view on actual risk to microorganisms and microbial processes.
3. The fact that larger changes in community structure that are induced by other types of management measures are accepted without any risk concerns for microbial community structure/diversity.
4. Recent guidance concludes that the requirement of studies on the effects of microbial biocontrol agents on microbial populations in soil are not motivated through assessments of risks of chemical plant protection products to in-soil organisms.
5. Populations of MBCA are generally decreasing after application. Potential subtle changes of community structures after MBCA application will thus last only for short periods.
6. Literature shows that long term effects on natural populations are rare and that recovery of natural populations does occur.
7. Potential changes of community structures after MBCA application are reported in a very limited number of reports as compared to the high number of reports on the use of MPCB and other beneficials (e.g. Trichoderma).

Based on the Sundh (2020) paper and the NL working group's endorsement and elaborations, the Ctgb successfully advocated for the removal of this data requirement for most microorganisms.

7 Decision tree IBMA (International Biocontrol Manufacturers Association)

The International Biocontrol Manufacturers Association (IBMA) proposed a decision tree, that was discussed in the NL working group. This decision tree is an attempt to discriminate among several groups of micro-organisms at an early stage in the risk assessment. For this purpose, this decision tree focuses on the biological properties of micro-organisms and their related risk areas. The decision tree starts with assigning the micro-organism under consideration to three categories of micro-organisms, i.e. obligate parasites; facultative, saprophyte, parasites; and non-systemic endosymbiont or true endophytes. The idea behind this division is that each of the 3 categories enters a separate part of the decision tree requiring the appropriate data for the risk assessment of that category.

The NL working evaluated the decision tree as an interesting approach. The working group discussed if all micro-organisms fall within one of the 3 categories and if the criteria between the 3 categories are that strict that this warrants a separate risk assessment. The Working Group had the opinion that this is not the case. An advantage of the decision tree is that it is scientifically underpinned and clearly stated in which cases data are or are not needed. However, what to do if the micro-organism does not fall within one of the 3 categories ? Also the working group is missing a “fall-back” option, i.e. a route which you can follow once you will have collected more information and data.

The decision tree discriminates between endophytes and epiphytes. The working group already discussed this topic intensively (see Chapter 4). Many micro-organisms are optionally endophytic. So, according to the Dutch scientific working group, absence of endophytic growth is not a good criterium to be applied in any guidance or decision tree.

In general, the working group advises to include more clarification of terms related to the three categories. There was also concern that mycotoxins would not be adequately assessed in the proposed decision tree. A possibility is to disqualify known producers of mycotoxins for use as plant protection product early in the decision tree.

8 Adapted uniform principles and data requirements and other topics

The new uniform principles and the new data requirements for microbial pesticides were accepted by the European Commission and came into force on 21 November 2022. For reference see: https://food.ec.europa.eu/plants/pesticides/micro-organisms_en.

The following four Regulations were implemented:

- Commission Regulation (EU) 2022/1438, amending Annex II to Regulation (EC) No 1107/2009 as regards specific criteria for the approval of active substances that are micro-organisms.
- Commission Regulation (EU) 2022/1439, amending Regulation (EU) No 283/2013 as regards the information to be submitted for active substances and the specific data requirements for micro-organisms.
- Commission Regulation (EU) 2022/1440, amending Regulation (EU) No 284/2013 as regards the information to be submitted for plant protection products and the specific data requirements for plant protection products containing micro-organisms.
- Commission Regulation(EU) 2022/1441, amending Regulation (EU) No 546/2011 as regards specific uniform principles for evaluation and authorisation of plant protection products containing micro-organisms.

Some other aspects of the risk assessment were discussed in a more general sense in the NL working group:

1. Waar gaat het over: onderwerp.
2. Wat is er voorgesteld in de docs under review.
3. En wat zijn de overwegingen van de WG hierbij.
4. Optioneel: advies voor verder onderzoek/guidance.

Topic: Inclusion of hazard-based approach
The approach of the adapted regulation is to assess hazard first. Based on the outcome, further risk assessment might be needed. This approach has been adopted in the new data requirements.

Topic: Addressing pathogenicity
The new approach is to collect data about potential pathogenicity first. Animal studies will only be required in a next step, if deemed necessary. So, the regulation was adapted to minimize the number of animal studies.

Topic: Assessment of residues
The hazard for any residues will be evaluated first. If a hazard has been identified, exposure needs to be addressed further.

Topic: Indigenousness of biocontrol micro-organisms
According to OECD guidelines the assessment of potential invasiveness is only required for non-indigenous micro-organisms. However, for microorganisms this is not straightforward. This topic was discussed in the Dutch scientific working group. The definition of 'indigenous' was not clear to the working group. Does this mean indigenous in the EU, or in a certain ecosystem? Secondly, what level of taxonomic classification is relevant regarding being indigenous? In the end, the term "indigenous" and related requirements were not included in the new data requirements.

Topic: Invasiveness of biocontrol micro-organisms
The Dutch scientific working discussed this topic. A microorganism is considered to be invasive when it is capable of spreading and growing in new ecosystems and exerts negative influence on local populations or ecosystems. There is little evidence for the occurrence of non-pathogenic, invasive microorganisms (Litchman, 2010). This is underpinned by available literature showing that populations of microorganisms used as MBCA generally decline upon application (several publications, see chapter 6). This population decline is assumed to occur due to the buffering capacity of soil microbial communities which outcompete the introduced microorganism. There are indications that the microbial diversity in soil is an important factor in

determining the survival of introduced microorganisms; the higher the diversity, the lower the survival rates (Van Elsas et al., 2012). This relationship could be explained by a decrease in the competitive ability of the invader in species-rich vs. species-poor bacterial communities. Therefore, soil microbial diversity is a key factor that controls the extent to which bacterial invaders can establish.

In order to assess potential adverse effects resulting from invasiveness of any MCPA, be it indigenous or not, several trait-related aspects have to be assessed e.g. the potential to proliferate in the environment, the potential to disperse to different ecosystems and potential adverse effects resulting from this dispersal. Examples of adverse effects can be suppression of microorganisms important for essential soil functions such as nutrient cycling and decomposition, thereby affecting soil fertility. These effects can only take place in case the MBCA has a selective advantage of high fitness in the soil ecosystem for example resulting from its antimicrobial properties or by its strong competitive abilities. All three aspects (proliferation, dispersal and fitness/selective advantage) can be assessed on a case-by-case basis and can be supported by experimental data, if considered necessary.

Ultimately, the topic of invasiveness has not been adopted as a criterium in the data requirements; part of the discussion about invasiveness was related to the question if the biocontrol micro-organisms are native. If a micro-organism is native and occurring in EU habitats relevant for agriculture, this will be evaluated within the required risk assessment. This approach was endorsed by the Dutch working group.

Whole genome sequencing

EFSA published a statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain (EFSA, 2021). (https://www.efsa.europa.eu/sites/default/files/2021-03/EFSA-statement-EFSA-Q-2019-00434.pdf) Microorganisms, genetically modified or not, may be used in the food chain as such or as production organisms of substances of interest. The placement of such microorganisms or derived substances/products in the European market may be subject to a pre-market authorization process. The authorization process defines the need to perform such a risk assessment. As a first step in the risk assessment, the microorganism/s subject to the application for authorization need to be characterized. In this regard, data obtained from whole genome sequence analysis can provide information on the unequivocal taxonomic identification of the strains and on the characterization of their potential functional traits of concern which may include virulence factors, resistance to antimicrobials of clinical relevance for humans and animals, production of known toxic metabolites and on the presence of genes coding for virulence, pathogenicity and/or toxigenicity. From this point of view, whole genome sequence analysis as a method for the evaluation of food safety, is also promising to be used for identifying the risks of microbial pesticides in the environment. In fact, in some areas of the regulated products the use of whole genome sequence-based data has already been established as a requirement for the risk assessment. This document provides recommendations to applicants on how to perform whole genome sequencing and the quality criteria/thresholds that should be reached as well as the data and relevant information that is needed (EFSA, 2021).

References

Cordier C, Alabouvette C, 2009. Effects of the introduction of a biocontrol strain of Trichoderma atroviride on non-target soil micro-organisms. European Journal of Soil Biology 45 (2009) 267–274EU, 2013 a. COMMISSION REGULATION (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. Section B. Micro-organisms including viruses. Official Journal of the European Commission. Pag. 65 – 84.

European Food Safety Authority (EFSA), 2021. EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain. EFSA Journal, 10 March.

Elsas, van, J.D., M. Chiurazzia, C.A. Mallona, D. Elhottova, V. Krištufek and J. Falcão Sallesa, 2012. Microbial diversity determines the invasion of soil by a bacterial pathogen. PNAS, 24, 109 (4): 1159–1164.

EU, 2013b. COMMISSION REGULATION (EU) No 284/2013 of 1 March 2013 setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. Section B. Micro-organisms including viruses. Official Journal of the European Commission. Pag. 92 e.v.

EC, 2020. Guidance on the risk assessment of metabolites produced by micro-organisms used as plant protection active substances in accordance with article 77 of Regulation (EC) no. 1107/2009. 42 pp.

EC, 2020. Guidance on the approval and low-risk criteria linked to the "antimicrobial resistance" applicable to micro-organisms used for plant protection in accordance with regulation (EC) 1107/2009. 14 pp.

Köhl, Jürgen, Kees Booij, Rogier Kolnaar, Willem J. Ravensberg, 2019. Ecological arguments to reconsider data requirements regarding the environmental fate of microbial biocontrol agents in the registration procedure in the European Union. BioControl (2019) 64:469–487. https://doi.org/10.1007/s10526-019-09964-y(0123456789().,-volV() 0123458697().,-volV).

Litchman, E., 2010. Invisible invaders: non-pathogenic invasive microbes in aquatic and terrestrial ecosystems. Ecology Letters, (2010) 13: 1560–1572.

OECD, 2018. Working Document on the Risk Assessment of Secondary Metabolites of Microbial Biocontrol Agents. Series on Pesticides No. 98. ENV/JM/MONO 33 pp.

Scheepmaker, J.W.A., M. Busschers, I. Sundh, J. Eilenberg and T.M. Butt, 2019. Sense and nonsense of the secondary metabolites data requirements in the EU for beneficial microbial control agents. Biological Control 136 (2019) 104005. https://doi.org/10.1016/j.biocontrol.2019.104005.

Scheepmaker, J.W.A., J. van de Kassteele (2011). Effects of chemical control agents and microbial biocontrol agents on numbers of non-target microbial soil organisms: a meta-analysis. Biocontrol Science and Technology 21(10), 1225 – 1242.

Scheepmaker, J.W.A. & F.M.W. de Jong, 2017. Vergroening door microbiële gewasbeschermingsmiddelen. Verkenning knelpunten en oplossingsrichtingen. RIVM Briefrapport 2017-0111. 96 pp.

Scheepmaker, J.W.A., 2021. Exploring the necessity of additional data requirements under the pesticide regulation to take into account endophytes. RIVM Letter report 2021-0056, 54 pp.

Sundh, I., 2020, On the need for determining effects of microbial control agents on (non-target) microbial communities in the environment. Paper collated for the 'Biopesticides' work group of DG SANTE, the EU Commission. Unpublished.

WHO (World Health Organization), 2015. Global action plan on antimicrobial resistance ISBN: 9789241509763. 28 pp.

WHO, 2019. Critically Important Antimicrobials for human medicine 6th revision 2018. https://apps.who.int/iris/bitstream/handle/10665/312266/9789241515528-eng.pdf?ua=1.

Wageningen Environmental Research
P.O. Box 47
6700 AA Wageningen
The Netherlands
T 0317 48 07 00
wur.eu/environmental-research

Report 3267
ISSN 1566-7197

To explore the potential of nature to improve the quality of life

Wageningen Environmental Research
P.O. Box 47
6700 AB Wageningen
The Netherlands
T +31 (0) 317 48 07 00
wur.eu/environmental-research

Report 3267
ISSN 1566-7197

The mission of Wageningen University & Research is "To explore the potential of nature to improve the quality of life". Under the banner Wageningen University & Research, Wageningen University and the specialised research institutes of the Wageningen Research Foundation have joined forces in contributing to finding solutions to important questions in the domain of healthy food and living environment. With its roughly 30 branches, 7,200 employees (6,400 fte) and 13,200 students and over 150,000 participants to WUR's Life Long Learning, Wageningen University & Research is one of the leading organisations in its domain. The unique Wageningen approach lies in its integrated approach to issues and the collaboration between different disciplines.